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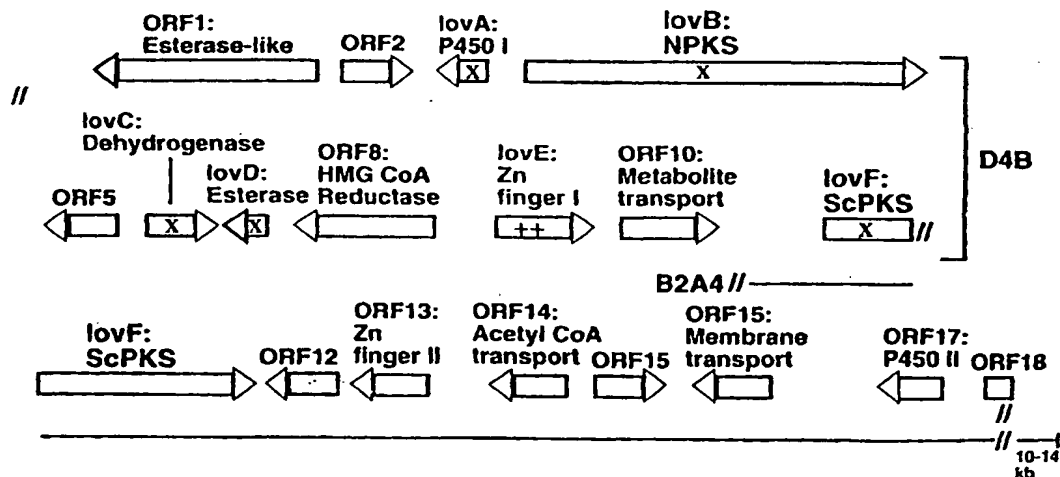


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(54) Title: METHOD OF PRODUCING ANTIHYPERCHOLESTEROLEMIC AGENTS

Lovastatin production genes



(57) Abstract

A method of increasing the production of lovastatin or monacolin J in a lovastatin-producing or non-lovastatin-producing organism is disclosed. In one embodiment, the method comprises the steps of transforming an organism with the *A. terreus* D4B segment, wherein the segment is translated and where an increase in lovastatin production occurs.

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METHOD OF PRODUCING ANTIHYPERCHOLESTEROLEMIC AGENTS

CROSS-REFERENCES TO RELATED APPLICATION

Not applicable.

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5 This invention was made with United States
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in this invention.

BACKGROUND OF THE INVENTION

10 Cholesterol and other lipids are transported in body
fluids by low-density lipoproteins (LDL) and high-density
lipoproteins (HDL). Substances that effectuate
mechanisms for lowering LDL-cholesterol may serve as
effective antihypercholesterolemic agents because LDL
15 levels are positively correlated with the risk of
coronary artery disease.

 MEVACOR (lovastatin; mevinolin) and ZOCOR
(simvastatin) are members of a group of active
antihypercholesterolemic agents that function by
20 inhibiting the rate-limiting step in cellular cholesterol
biosynthesis, namely the conversion of
hydroxymethylglutarylcoenzyme A (HMG-CoA) into mevalonate
by HMG-CoA reductase.

 The general biosynthetic pathway of a naturally
25 occurring HMG-CoA reductase inhibitor has been outlined
by Moore, et al., who showed that the biosynthesis of

mevinolin (lovastatin) by *Aspergillus terreus* ATCC 20542 begins with acetate and proceeds via a polyketide pathway (R.N. Moore, et al., J. Amer. Chem. Soc. 107:3694-3701, 1985). Endo, et al. described similar biosynthetic
5 pathways in *Penicillium citrinum* NRRL 8082 and *Monascus ruber* M-4681 (A.Y. Endo, et al., J. Antibiot. 38:444-448, 1985).

The recent commercial introduction of microbial HMG-CoA reductase inhibitors has fostered a need for high
10 yielding production processes. Methods of improving process yield have included scaling up the process, improving the culture medium and simplifying the isolation.

Previous attempts to increase the biosynthesis of
15 HMG-CoA reductase inhibitors at the level of gene expression have focused on increasing the concentration triol polyketide synthase (TPKS), a multifunctional protein with at least six activities as evidenced by the product of the enzymatic activity (Moore, supra, 1985).
20 TPKS is believed to be the rate-limiting enzymatic activity(ies) in the biosynthesis of the HMG-CoA reductase inhibitor compounds.

U.S. patent 5,744,350 identifies a DNA encoding triol polyketide synthase (TPKS) from *Aspergillus*
25 *terreus*. "NPKS" is now preferred to TPKS as the acronym for "nonaketide polyketide synthase."

SUMMARY OF THE INVENTION

In one embodiment, the present invention is a method of increasing the production of lovastatin in a lovastatin-producing organism. The method comprises the steps of transforming the organism with a nucleic acid sequence comprising the D4B segment, preferably comprising nucleotides 579 - 33,000 of SEQ ID NO:18 and 1 - 5,349 of SEQ ID NO:19. The nucleic acid sequence is transcribed and translated and an increase in lovastatin production occurs. Preferably, this increase is at least 2-fold.

In a preferred form of the present invention, the lovastatin-producing organism is selected from the group consisting *A. terreus* ATCC 20542 and ATCC 20541.

In another embodiment, the method comprises the step of transforming the organism with the corresponding D4B segment isolated from a non-*A. terreus* lovastatin-producing organism.

In another embodiment, the present invention is a method of increasing the production of lovastatin in a lovastatin-producing organism, comprising the step of transforming the organism with the *LovE* gene, wherein the nucleic acid sequence is transcribed and translated and wherein an increase in lovastatin production occurs.

In another embodiment of the present invention, one may increase the production of monacolin J in a non-lovastatin-producing organism comprising the steps of

transforming the organism with a nucleic acid sequence comprising the D4B segment. As a further step, one may additionally transform the organism with an entire LovF gene. If the entire LovF gene is added to the D4B
5 segment, the organism will produce lovastatin.

In another embodiment, the present invention is the lovastatin production gene cluster, preferably SEQ ID NOs:18 and 19, and the individual genes comprising that cluster.

10 It is an object of the present invention to provide a method for increasing lovastatin and monacolin J production in both lovastatin-producing and non-lovastatin producing organisms.

Other objects, features and advantages of the
15 present invention will become apparent after review of the specification, claims and drawings.

DESCRIPTION OF DRAWINGS

Fig. 1 is a diagram of lovastatin production genes.

Fig. 2 is a schematic diagram of a hypothetical
20 mevinolin/lovastatin biosynthesis pathway.

Fig. 3 is a comparative diagram of statins.

Fig. 4 is a schematic drawing of plasmid
pWHM1264/CB24A.

Fig. 5 is a schematic drawing of plasmid pWHM1424.

25 Fig. 6 is a schematic drawing of plasmid
CD4B/pWHM1263.

DESCRIPTION OF THE INVENTION

In General

The Examples below disclose the cloning and sequencing of a cluster of 17 genes from *A. terreus* ATCC 20542, a strain that natively produces lovastatin (See Fig. 1). These genes flank the NPKS gene, which is known to be required for lovastatin production (see, for example, U.S. patent 5,744,350).

The DNA sequence of the cluster has been determined and is disclosed below at SEQ ID NOs:18 and 19. Mutations in four of the genes (P450I/LovA, SEQ ID NO:22; dehydrogenase/LovC, SEQ ID NO:24; esterase/LovD, SEQ ID NO:25; and ScPKS/LovF, SEQ ID NO:29) have been isolated and demonstrate that each of these four individual genes is required for lovastatin production. These genes are indicated with an X symbol in Fig. 1 and referred to herein as the "*A. terreus* lovastatin gene cluster."

Another of the genes (Zn Finger I/LovE, SEQ ID NO:27) is thought to regulate the transcription of the other genes and causes a notable increase in lovastatin production when reintroduced into *A. terreus* ATCC 20542.

Applicants have used the following convention in naming the genes and proteins of the present invention. The genes and proteins are first named with either an "ORF" or "Lov" prefix and then named either numerically or alphabetically. "Lov" signifies genes shown to be essential for lovastatin production. Applicants have

also included a descriptor name that describes a probable function of the protein. For example, SEQ ID NO:1 is described as the "ORF1/esterase-like protein" because Applicants have compared the amino acid sequence to known
5 esterases.

The portion of the gene cluster between ORF1/esterase-like protein and the mid-region of LovF/SCP_{KS} is referred to as the "D4B segment". The *A. terreus* D4B segment is contained within a plasmid clone
10 deposited as ATCC 98876. As described below, other lovastatin-producing organisms contain an analogous D4B segment comprising analogous genes. The present invention comprises a "D4B segment" isolated from other lovastatin-producing organisms. The arrangement of the
15 genes within the D4B segment may be different in other organisms. We predict that the genes within these other segments will have at least 80% homology, at the nucleic acid level, with the genes disclosed herein. We envision that each of these lovastatin-producing organisms will
20 comprise within their genomes a LovA, LovB, LovC, LovD, LovE and LovF gene.

We have determined that the D4B segment will confer production of monocolin J if the genes are all expressed, as we show below in an example using *A. nidulans*. We
25 envision that adding the LovF gene to the D4B segment genes will result in the production of lovastatin in a non-lovastatin-producing organism.

Table 1, below, summarizes information regarding the different protein and nucleic acid sequences of the present invention. SEQ ID NOs:1-17 are predicted translation products of various members of the gene cluster. SEQ ID NOs:18 and 19 are the entire DNA sequence of the gene cluster. SEQ ID NOs:21-36 are the genomic DNA sequences of the various members of the gene cluster and include the introns. These DNA sequences are reported in the Sequence Listing in the 5' - 3' orientation, although, as Fig. 1 indicates, some of these DNA sequences are in the inverted orientation in the actual cluster.

TABLE 1

SEQ ID NO.	DESCRIPTION	COMMENTS
SEQ ID NO: 1	Predicted amino acid sequence of ORF1/Esterase-like protein	Translation of 6 EXONS 6865-6568, 6462-5584, 5520-4822, 4774-3511, 3332-2372, 2301-1813 (reverse complement) FROM SEQ ID NO:18
SEQ ID NO: 2	Predicted amino acid sequence of ORF2	Translation of 1 EXON 7616-8602 FROM SEQ ID NO:18
SEQ ID NO: 3	Predicted amino acid sequence of LovA/P4501 protein	Translation of 1 EXON 10951-9980 (reverse complement) FROM SEQ ID NO:18
SEQ ID NO: 4	Predicted amino acid sequence of ORF5	Translation of 1 EXON 22760-21990 (reverse complement) FROM SEQ ID NO:18
SEQ ID NO: 5	Predicted amino acid sequence of LovC/Dehydrogenase	Translation of 3 EXONS 23158-23717, 23801-23912, 23991-24410 FROM SEQ ID NO:18
SEQ ID NO: 6	Predicted amino acid sequence of LovD/Esterase	Translation of 3 EXONS 26203-26080, 26005-25017, 24938-24810 (reverse complement) FROM SEQ ID NO:18

	SEQ ID NO.	DESCRIPTION	COMMENTS
	SEQ ID NO: 7	Predicted amino acid sequence of ORF8/HMG CoA Reductase	Translation of 5 EXONS 30062-29882, 29803-29745, 29664-27119, 27035-26779, 26722-26559 (reverse complement) FROM SEQ ID NO:18
	SEQ ID NO: 8	Predicted amino acid sequence of LovE/Zn Finger I	Translation of 1 EXON 31360-32871 FROM SEQ ID NO:18
	SEQ ID NO: 9	Predicted amino acid sequence of ORF10/Metabolite transport	Translation of 8 EXONS 1400-1452, 1619-1695, 1770-1996, 2065-2088, 2154-2225, 2332-2865, 2939-3099, 3180-3560 FROM SEQ ID NO:19
	SEQ ID NO: 10	Predicted amino acid sequence of LovF/ScPKS	Translation of 7 EXONS 4430-4627, 4709-4795, 4870-4927, 4985-5318, 5405-5912, 5986-6565, 6631-12464 FROM SEQ ID NO:19
5	SEQ ID NO: 11	Predicted amino acid sequence of ORF12	Translation of 3 EXONS 13596-13496, 13451-13063, 12968-12709 (reverse complement) FROM SEQ ID NO: 19
	SEQ ID NO: 12	Predicted amino acid sequence of ORF13/Zn Finger II	Translation of 5 EXONS 16608-16463, 16376-15572, 15519-15346, 15291-14825, 14767-14131 (reverse complement) FROM SEQ ID NO: 19
	SEQ ID NO: 13	Predicted amino acid sequence of ORF14/Acetyl CoA transport protein	Translation of 7 EXONS 19642-19571, 19502-19427, 19352-19227, 19158-19011, 18956-18663, 18587-18438, 18380-18341 (reverse complement) FROM SEQ ID NO:19
	SEQ ID NO: 14	Predicted amino acid sequence of ORF15	Translation of 2 EXONS 20332-20574, 20631-21860 FROM SEQ ID NO:19
	SEQ ID NO: 15	Predicted amino acid sequence of ORF16/Membrane transport protein	Translation of 5 EXONS 24521-24054, 23996-23936, 23876-23184, 23111-22977, 22924-22818 (reverse complement) FROM SEQ ID NO:19
10	SEQ ID NO: 16	Predicted amino acid sequence of ORF17/P450II protein	Translation of 3 EXONS 28525-27673, 27606-27284, 27211-26837 (reverse complement) FROM SEQ ID NO:19

SEQ ID NO.	DESCRIPTION	COMMENTS
SEQ ID NO: 17	Predicted amino acid sequence of ORF18 (incomplete)	Translation of 2 EXONS 29826-30995, 31054-31328 (incomplete) FROM SEQ ID NO:19
SEQ ID NO: 18	DNA sequence of gene cluster-first 33,000 nucleotides	
SEQ ID NO: 19	DNA sequence of cluster-nucleotides 33,001-64,328 (renumbered 1-31,328)	
SEQ ID NO: 20	DNA sequence of ORF1/Esterase-like gene	Start = 6865 Stop = 1813 SEQ ID NO:18
5 SEQ ID NO: 21	DNA sequence of ORF2	Start = 7616 Stop = 8602 SEQ ID NO:18
SEQ ID NO: 22	DNA sequence of LovA/P450I gene	Start = 10951 Stop = 9980 SEQ ID NO:18
SEQ ID NO: 23	DNA sequence of ORF5	Start = 22760 Stop = 21990 SEQ ID NO:18
SEQ ID NO: 24	DNA sequence of LovC/Dehydrogenase	Start = 23158 Stop = 24410 SEQ ID NO:18
SEQ ID NO: 25	DNA sequence of LovD/Esterase	Start = 24810 Stop = 26203 SEQ ID NO:18
10 SEQ ID NO: 26	DNA sequence of ORF8/HMG CoA Reductase	Start = 30062 Stop = 26559 SEQ ID NO:18
SEQ ID NO: 27	DNA sequence of LovE/Zn Finger I	Start = 31360 Stop = 32871 SEQ ID NO:18
SEQ ID NO: 28	DNA sequence of ORF10/Metabolite transport	Start = 1400 Stop = 3560 SEQ ID NO:19
SEQ ID NO: 29	DNA sequence of LovF/ScPKS	Start = 4430 Stop = 12464 SEQ ID NO:19
SEQ ID NO: 30	DNA sequence of ORF12	Start = 13596 Stop = 12709 SEQ ID NO:19

SEQ ID NO.	DESCRIPTION	COMMENTS
SEQ ID NO: 31	DNA sequence of ORF13/Zn Finger II	Start = 16608 Stop = 14131 SEQ ID NO:19
SEQ ID NO: 32	DNA sequence of ORF14/Acetyl CoA transport gene	Start = 19642 Stop = 18341 SEQ ID NO:19
SEQ ID NO: 33	DNA sequence of ORF15	Start = 20332 Stop = 21860 SEQ ID NO:19
SEQ ID NO: 34	DNA sequence of ORF16/Membrane transport protein	Start = 24521 Stop = 22818 SEQ ID NO:19
5 SEQ ID NO: 35	DNA sequence of ORF17/P450II gene	Start = 28525 Stop = 26837 SEQ ID NO:19
SEQ ID NO: 36	DNA sequence of ORF18 (incomplete)	Start = 29826 to 31328 (incomplete) SEQ ID NO:19

Table 1 also notes the translation start and stop points in the various gene sequences.

10 The sequence of the NPXS gene is not listed in SEQ ID NOs:21-36. This gene is characterized in U.S. patent 5,744,350. However, SEQ ID NOs:18 and 19 do contain the sequence of the NPXS gene within the context of the entire gene cluster.

15 To perform many embodiments of the present invention, one will need to recreate various genes or a portion of the gene cluster described herein. Applicants have provided sequence data in the Sequence Listing sufficient to allow one of skill in the art to construct
20 numerous probes suitable to recreate the genes from an *A. terreus* genomic library. Applicants have also described below various methods for isolating *A. terreus* DNA.

Additionally, Applicants have deposited ATCC
Accession No. ATCC 98876, which contains clone pWHM1263
(CD4B) and ATCC Accession No. ATCC 98877 which contains
clone pWHM1265 (CB2A4). Both plasmids are described in
5 more detail below. Fig. 4 describes clone
CB2A4/pWHM1265, and Fig. 6 describes clone CB4B/pWHM1263.
Fig. 1 also indicates the boundaries of the D4B and B2A4
clones.

The clones and their inserts may be prepared from
10 the ATCC deposits by methods known to those of skill in
the art. The DNA from the clones may be isolated and any
gene within the gene cluster may be isolated and
utilized.

15 Increasing the Production of Lovastatin by Lovastatin-
producing Fungi and Yeast

In one embodiment, the present invention is a method
of increasing the production of lovastatin in a
lovastatin-producing fungi and yeast, preferably *A.*
terreus ATCC20542 and ATCC20541. Other examples of
20 suitable lovastatin-producing fungi and yeast are listed
in Table 2, below.

TABLE 2

<u>Microorganisms other than <i>A. terreus</i> reported to produce lovastatin (mevinolin)</u>	
	Monascus (17 of 124 strains screened) species ¹
5	M. ruber M. purpureus M. pilosus M. vitreus M. pubigerus
10	Penicillium sp. ¹ Hypomyces sp. Doratomyces sp. Phoma sp. Eupenicillium sp.
15	Gymnoascus sp. Trichoderma sp.
	Pichia labacensis ² Candida cariosilognicola
20	Aspergillus oryzae ³ Doratomyces stemonitis Paecilomyces virioli Penicillium citrinum Penicillium chrysogenum Scopulariopsis brevicaulis
25	Trichoderma viride
30	1. P. Juzlova, L. Martinkova, V. Kren. Secondary Metabolites of the fungus Monascus: a review. <u>J. Ind. Microbiol.</u> 16:163-170 and references cited therein (1996). 2. N. Gunde-Cimerman, A. Plemenitas and A. Cimerman. A hydroxymethylglutaryl-CoA reductase inhibitor synthesized by yeasts. <u>FEMS Microbiol. Lett.</u> 132:39-43 (1995). 3. A.A. Shindia. Mevinolin production by some fungi. <u>Folia Microbiol.</u> 42:477-480 (1997).

By "increasing the production" we mean that the amount of lovastatin produced is increased by at least 2-fold, preferably by at least 5-fold. The examples below demonstrate two preferred methods for analyzing strains for lovastatin production. In method A, the spore suspension is inoculated into a flask of SEED medium and grown. The resulting seed culture is used to inoculate FM media and grown for six days. In fermentation method

B, one inoculates 50 ml of RPM media and grows this larger culture for 7 days.

Both cultures are extracted, pH adjusted, mixed with ethyl acetate and shaken for two hours. For analysis, 1
5 ml of the ethyl acetate layer is dried under a nitrogen stream and resuspended in methanol. For TLC analysis, a small amount of the extract is run on C18 reverse phase TLC plates in a solvent system of methanol; 0.1% phosphoric acid. The TLC plates are developed by
10 spraying with phosphomolybdic acid in methanol and heating with a heat gun. The extracts are compared with authentic lovastatin, monacolin J, monacolin L and dihydromonoclon L.

If one wishes HPLC analysis, the examples below
15 describe the use of a Waters Nova-Pak C18 column used with a solvent system of acetonitrile and phosphoric acid. A Waters 996 Photodiode Array Detector will detect the metabolites. Lovastatin was detected at 238 nm.

In one embodiment, one would transform a lovastatin-
20 producing fungi or yeast with the lovE/zinc finger I gene, preferably comprising the nucleotides of SEQ ID NO:27. The examples below predict that this will result in an increase of at least 5-7 fold. Preferably, the increase will be at least 2.0-fold.

25 One may also transform a lovastatin-producing fungi or yeast with the LovE gene isolated from other lovastatin-producing fungi or yeast. One may obtain this

gene by use of a probe derived from SEQ ID NO:27 by methods known to those of skill in the art.

One may also transform lovastatin-producing fungi and yeast with the D4B segment of the lovastatin
5 production gene cluster (see Fig. 1), preferably as found in ATCC accession number 98876. Alternatively, one may transform lovastatin-producing fungi or yeast with the entire gene cluster, as diagramed in Fig. 1.

We envision that to successfully increase lovastatin
10 production, one may also wish to transform less than the entire gene cluster. Preferably, one may determine what the smallest possible segment is by deleting various portions of the gene cluster and determining whether lovastatin production is continually increased.
15 Similarly, if one begins with the D4B segment, one may delete various portions for the segment and determine whether lovastatin production is continually increased by at least 2-fold.

Modification of the LovB/NPKS gene would produce
20 other HMG CoA inhibitors. For example, Fig. 3 diagrams the relationship between mevastatin, lovastatin, simvastatin and pravastatin. In one example, the methyl transferase domain of the NPKS gene may be replaced with an inactive form to make pravastatin. The HMG-CoA
25 reductase inhibitors within this invention include, but are not limited to, compactin (ML-236B), lovastatin, simvastatin, pravastatin and mevastatin.

In another embodiment of the present invention, one may transform a lovastatin-producing organism with the genes described above and obtain the production of an HMG CoA reductase inhibitor with a structure different from monacolin J, monacolin L or lovastatin. Alterations in the side chain attached to C8 are the most likely possibility but other alterations may occur. These alterations would happen through the native biochemistry of the organism.

10 If one wishes to express the *A. terreus* genes in yeast, one may wish to consult examples in which others have engineered fungal secondary metabolism genes for expression in yeast. (See for example, J. T. Kealey, et al., Proc. Natl. Acad. Sci. USA 95:505-509 (1998)). The exact approach could be used with the NPKS (LovB) and ScpKS (LovF) genes, and a somewhat simpler approach with the other lovastatin genes in their cDNA forms.

Production of HMG-CoA Reductase Inhibitors by Fungi and Yeast That Do Not Natively Produce Inhibitors.

20 In another embodiment, the present invention is the production of HMG-CoA reductase inhibitors, such as lovastatin, by fungi and yeast that do not natively produce lovastatin. An example of a suitable fungi or yeast is *A. nidulans* and *S. cerevisiae*, respectively.

25 For this embodiment one preferably transforms the genes within the D4B segment into the non-inhibitor-producing strain. By this method, one would produce

monacolin J (See Fig. 2) which could be chemically converted to lovastatin by one of skill in the art.

Monacolin J, in its lactone form obtained by treatment with anhydrous acid under dehydrative conditions, is preferably treated with a derivative of (2S)-2-methylbutyric acid, in which the carboxyl group has been suitably activated for undergoing esterification, and the resulting lovastatin is isolated by conventional methods. For example, see WO 33538, U.S. patent 4,444,784 and J. Med. Chem. 29:849 (1986). These are citations for synthesis of simvastatin from monacolin J. One would use the same method, but use the (2S)-2-methylbutyrate derivative to make lovastatin.

In another embodiment of the present invention, one would transform the genes within the D4B segment, including an entire LovF/SCPXS gene, into the non-inhibitor-producing organism. By this method, one would produce lovastatin in a non-lovastatin-producing organism.

In another embodiment of the present invention, one may transform a non-lovastatin-producing organism with the genes described above and obtain the production of an HMG CoA reductase inhibitor with a structure different from monacolin J, monacolin L or lovastatin, as described above.

Modification of the LovB/NPKS gene would produce other inhibitors. For example, Fig. 3 diagrams the relationship between mevastatin, lovastatin, simvastatin

and pravastatin. In one example, the methyl transferase domain of the NPXS gene may be replaced with an inactive form to make pravastatin. The HMG-CoA reductase inhibitors within this invention include, but are not
5 limited to, compactin (ML-236B), lovastatin, simvastatin, pravastatin and mevastatin.

Production of Intermediate Materials

In another embodiment, the present invention is a method of isolating intermediate materials in the
10 production of lovastatin and analogs such as mevastatin and simvastatin. For example, the Examples below demonstrate the disruption of the lovastatin projection gene cluster with mutagenized LovC, LovD, LovF, LovA or LovB genes. Disruption of many of these genetic elements
15 of the lovastatin production gene cluster will result in accumulation of intermediate materials. Therefore, to practice this embodiment of the present invention, one would transform a suitable lovastatin-producing host with a mutagenized gene within the D4B segment, as described
20 below.

Many other mutations would be suitable to destroy the function of LovC, LovD, LovF, LovA or LovB. All that is necessary is these genes be disrupted to the extent that they are non-functional.

25 Production of Lovastatin Analogs

In another embodiment, the present invention provides a method for engineering the production of

lovastatin analogs in such organisms as fungi or yeast, using monacolin J as the starting point.

Isolated DNA Segments

In another embodiment, the present invention is a
5 DNA segment capable of conferring lovastatin or monacolin J production or increase in lovastatin or monacolin J production in yeast or fungi. In a preferred example, this segment is the "D4B segment" that is deposited at ATCC 98876. The nucleotide sequence of this segment is
10 found in residues 579 - 33,000 of SEQ ID NO:18 and residues 1 - 5,349 of SEQ ID NO:19.

In another embodiment, the present invention is the entire *A. terreus* lovastatin gene cluster, as exemplified by SEQ ID NOs:18 and 19 and ATCC deposits 98876 and
15 98877.

The present invention is also the individual genes that make up the *A. terreus* lovastatin gene cluster. Therefore, the present invention is a nucleic acid segment selected from the group of consisting of SEQ ID
20 NOs:20 - 36. Preferably, the present invention is the coding region found within SEQ ID NOs:20 - 36 and described in Table 1. The present invention is also a mutagenized version of SEQ ID NOs:22, 24, 25 and 29, wherein the gene is mutagenized to be non-functional in
25 terms of lovastatin or monacolin J production.

Organisms with Increased Lovastatin or Monacolin J Production

In another embodiment, the present invention are the organisms described above. These organisms include
5 lovastatin-producing organisms, preferably yeast and fungi, that have been engineered to display at least a 2-fold increase in lovastatin or monacolin J production. The organisms also include non-lovastatin-producing organisms, preferably yeast or fungi, that have been
10 engineered to produce monacolin J or lovastatin.

Antifungal Compounds

Applicants note that lovastatin, monocolin J, monocolin L and dihydromonocolin L all have varying degrees of antifungal activity. Applicants envision that
15 the present invention is also useful for providing antifungal compounds and organisms engineered to express antifungal compounds. Preferably, one would measure the antifungal properties of a compound in the manner of N. Lomovskaya, et al., Microbiology 143:875-883, 1997.
20 Measurement of inhibition of yeast growth can be found in R. Ikeura, et al., J. Antibiotics 41:1148, 1988. The same general methods could be used for all fungi. Both of these references are hereby incorporated by reference.

EXAMPLES

1. General Methods and ProceduresConstruction of an *A. terreus* ATCC20542 genomic library.

A. terreus ATCC20542 genomic DNA was partially
5 digested with *Sau*3AI so as to produce an average fragment
size of 40 - 50 kb. The partially digested genomic DNA
was then separated on a sucrose gradient and the 40 - 50
kb fraction was collected. Cosmid AN26 (Taylor and
Borgmann, Fungal Genet. Newsletter 43, 1996) was prepared
10 by digestion with *Cla*I, dephosphorylated with CIP, then
digested with *Bam*HI to create the two cosmid arms.
Ligation reactions with genomic DNA fragments and cosmid
arms were optimized and packaged using Gigapack III XL
packaging extract (Stratagene). The packaged cosmid
15 library was infected into *E. coli* JM109 and plated out
onto LB agar (Sambrook, et al., Molecular Cloning. A
Laboratory Manual. 2nd ed. Cold Spring Harbour
Laboratory Press, 1989; other standard methods used can
be found here also) with ampicillin (50 μ g/ml) plates.
20 After checking for the presence of insert DNA in a
selection of clones, 5000 colonies were picked into LB
plus 50 μ g/ml ampicillin filled microtitre plates and
grown overnight at 37°C. The colonies were replica
plated onto nylon membranes (Amersham Hybond-N).
25 Glycerol was added at a final concentration of 15% (v/v)
to the microtitre plates and these were stored at -70°C.

Isolation of genomic clones containing the lovastatin biosynthesis cluster.

A 2.8 kb *EcoRI* fragment from pTPKS100 containing part of the NPKS gene (Vinci, et al., U.S. Patent No. 5,744,350) was gel-isolated and labelled with digoxigenin using the Genius Kit II (Boehringer Mannheim). This labelled fragment was hybridized (65°C, 5x SSC) with the nylon membranes containing the *A. terreus* genomic library, then washed (65°C, 0.1x SSC). Two positive clones were identified, pWHM1263 (cD4B) and pWHM1264 (cJ3A). Two of these clones, pWHM1263 (cD4B) and pWHM1265 (cB2A4), have been deposited in the ATCC (American Type Culture Collection, 10801 University Boulevard, Menassas, VA 20110) at accession number ATCC 98876 and 98877, respectively, under the terms and conditions of the Budapest Treaty. The presence of the NPKS gene was confirmed initially by restriction digestion and later by DNA sequencing.

Overlapping clones were found by repeating the hybridization process using labelled fragments from both ends of the insert in pWHM1263. This resulted in the isolation of pWHM1265-1270 (cB2A4, cL3E2, cJ3B5, cO2B5, cR3B2, cW3B1) from downstream of the NPKS gene and pWHM1271 (cQ1F1) from upstream of NPKS. All these clones were transformed into *E. coli* strain STBL2 (Stratagene) to help prevent rearrangements.

Fig. 4 is a diagram of the cB2A4/pWHM1265 clone. This clone contains an insert of approximately 43 kb in

AN26 and includes the nucleotide sequence from at least nucleotides 4988 of SEQ ID NO:19 to nucleotide 31,328 of SEQ ID NO:19 and 10 - 14 kb of uncharacterized DNA. Fig. 6 is a schematic diagram of cD4B/pWHM1263. This clone contains a 37,770 bp insert in AN26 and contains nucleotides 579 - 33,000 of SEQ ID NO:18 and nucleotides 1 - 5,349 of SEQ ID NO:19.

Sequencing strategy and analysis.

A series of overlapping subclones (pWHM1272-pWHM1415) were constructed in pSPORT1 (Gibco-BRL) and pGEM3 (Promega). Plasmid DNAs for sequencing were prepared using the QiaPrep spin miniprep kit (Qiagen). Cycle sequencing was carried out using the AmpliTaq FS or BigDye reagents (ABI) and were analyzed using a ABI model 373 or 377 DNA Sequencer. Primer walking was performed by synthesis of 18-22 bp oligonucleotide primers based on the sequenced DNA strand, with the help of the Oligo 4.05 program (National Biosciences, Inc.). Every region of DNA was sequenced at least once on both strands. Direct sequencing of cosmids and PCR products was used to confirm adjoining regions where no overlapping clones existed. DNA sequence analysis and manipulations were performed using SeqMan (DNASTAR) and SeqEd (ABI) software. Assignments of putative ORFS, including putative introns, were performed with the aid of BLAST 2.0 searches (Atschul, et al., Nucl. Acids Res. 25:3389-3402, 1997), and the Genetics Computer Group (GCG) programs (Program Manual for the Wisconsin Package,

Version 8, September 1994, Genetics Computer Group,
Madison, WI), version 8.1.

Isolation and characterization of *lovF* (ScPKS, ORF11),
lovD (EST1, ORF7), *lovC* (DH, ORF6), and *lovA* (P450I,
5 ORF3) mutants.

lovF

To disrupt the polyketide synthase gene, *lovF*, a 1.7
kb *EcoRI* fragment internal to the *lovF* gene was subcloned
from pWHM1265 into pSPORT1 to give pWHM1291. The ScPKS
10 fragment was then subcloned from this vector, as an
Acc65I - *HindIII* fragment, into pPLOA (Vinci, et al.,
U.S. Patent No. 5,744,350) to give pWHM1416. This vector
contains the phleomycin (Zeocin, obtained from
Invitrogen) resistance gene for selection in *A. terreus*.
15 *A. terreus* ATCC20542 was then transformed to Zeocin
resistance with this plasmid as described below.
Transformants were screened for lovastatin production as
described below (Method A). In one of the transformants,
WMH1731, lovastatin production was abolished and a new
20 compound accumulated. This new compound comigrated with
monacolin J on TLC and HPLC according to the methods
described below. Semi-preparative HPLC was used to
partially purify the major product which was then
analyzed by HPLC - MS. The same mass and fragmentation
25 pattern as authentic monacolin J was observed. To
confirm the disruption of the *lovF* gene, total genomic
DNA was prepared from wild-type *A. terreus* ATCC20542 and
the WMH1731 mutant strain. The genomic DNA was digested

with *Bam*HI and *Hind*III, electrophoresed on an agarose gel and capillary blotted onto a nylon membrane. The membrane was hybridized with the 1.7 kb *Eco*RI fragment from pWHM1416 labelled using the Genius II kit

5 (Boehringer Mannheim) using the conditions described previously. The wild-type strain had hybridizing bands at 4.2 kb for *Bam*HI and 11.5 kb for *Hind*III. As predicted, the WMH1731 mutant strain had hybridizing bands at 6.5 kb and 2.2 kb for *Bam*HI and 11 kb and 7.8 kb

10 for *Hind*III confirming the homologous integration of a single copy of pWHM1416 at the *lovF* locus.

lovD

To disrupt the putative esterase/carboxypeptidase-like gene, *lovD*, a 4.8 kb *Not*I - *Eco*RI fragment from

15 pWHM1263 was subcloned into pSPORT1 to give pWHM1274. This plasmid was digested with *Hind*III and *Bsi*WI and a 1.8 kb fragment was isolated. The plasmid was also digested with *Hind*III and *Bam*HI and the 6.6 kb fragment was isolated. pPLOA was digested with *Bam*HI and *Acc*65I

20 and the 2.1 kb fragment containing the phleomycin resistance marker was purified. These three fragments were ligated together and used to transform competent *E. coli* cells. The expected plasmid, pWHM1417, containing the phleomycin resistance gene flanked by the beginning

25 and the end of the *lovD* gene was isolated. This plasmid was linearized by digestion with *Xba*I or *Rsr*II before

being used to transform *A. terreus* ATCC20542 to Zeocin resistance. Transformants were screened for lovastatin production as described below (Method A). In one of the transformants, WMH1732, lovastatin production was
5 abolished and a new compound accumulated. This new compound comigrated with monacolin J on TLC and HPLC according to the methods described below. Semi-preparative HPLC was used to partially purify the major product which was then analyzed by HPLC - MS. The same
10 mass and fragmentation pattern as authentic monacolin J was observed. To confirm the disruption of the *lovD* gene, total genomic DNA was prepared from wild type *A. terreus* ATCC20542 and the WMH1732 mutant strain. The genomic DNA was digested with *ApaI*, run out on an agarose
15 gel and capillary blotted onto a nylon membrane. The membrane was hybridized with the 4.8 kb *NotI* - *EcoRI* fragment from pWHM1274 labelled using the Genius II kit using the conditions described previously. The wild-type strain had hybridizing bands at 9 kb, 8.4 kb and 1.5 kb.
20 As predicted the mutant strain had hybridizing bands at 9 kb, 8 kb, 3 kb and 1.5 kb confirming the homologous integration of a single copy of pWHM1417 at the *lovD* locus.

lovA

25 To disrupt the cytochrome P450 I gene, *lovA*, an 11 kb *Acc65I* - *EcoRI* fragment from pWHM1263 was subcloned into pGEM3 to give pWHM1272. From this plasmid a 2.1 kb

ApaI - SnaBI fragment was purified and ligated to ApaI - EcoRV digested pPLOA to give p450Phleo (pWHM1418). From this plasmid a 4.2 kb ApaI - NotI fragment was purified and ligated with a 1.8 kb EagI - KpnI fragment from pWHM1272 and ApaI - KpnI digested pGEM7 to give p450Dphleo (pWHM1419) which contains the *lovA* gene disrupted by the phleomycin resistance gene. This plasmid was then digested with KpnI and ApaI and the resulting fragment was used to transform *A. terreus* ATCC20542 to Zeocin resistance. Transformants were screened for lovastatin production as described below (Method A). In one of the transformants, WMH1733, lovastatin production was abolished and two new compounds accumulated. Genomic DNA was prepared from this strain and from *A. terreus* ATCC20542, digested with EagI, run out on an agarose gel, and capillary blotted onto a nylon membrane. The membrane was hybridized with the 6 kb ApaI - KpnI fragment from pWHM1419 labelled using the Genius II kit using the conditions described previously. The wild-type strain had hybridizing bands at 2.0 kb, 1.9 kb and 1.1 kb. Mutant strain WMH1733 had hybridizing bands at 2.5 kb, 2.0 kb, 1.1 kb and 0.7 kb confirming the homologous integration of a single copy of the fragment from pWHM1419 at the *lovA* locus.

lovC

To disrupt the dehydrogenase-like gene, *lovC*, a 2 kb *EcoRI* - *BglII* fragment from pTPKS100 was ligated with a 1.7 kb *EcoRI* - *SacI* fragment from pWHM1274 and *BglII* - *SacI* digested litmus 28 (New England Biolabs) to produce pDH1 (pWHM1420). Another plasmid pDH2 (pWHM1421) was constructed from a 2.2 kb *Acc65I* - *SacI* fragment from pWHM1274, a 2.1 kb *HindIII* - *SacI* fragment from pPLOA containing the phleomycin resistance gene and *HindIII* - *Acc65I* digested litmus 28. The disruption vector pDH-dis (pWHM1422) was constructed by ligating together a 2.5 kb *BglII* - *HpaI* fragment from pWHM1420, a 4.3 kb *EcoRV* - *KpnI* fragment from pWHM1421 and *BglII* - *KpnI* digested litmus 28. This plasmid was digested with *BglII* and *KpnI* and the resulting 6.8 kb fragment was used to transform *A. terreus* ATCC20542 to Zeocin resistance. Transformants were screened for lovastatin production as described below (Method A). In two of the transformants, WMH1734 and WMH1735, lovastatin production was abolished.

Genomic DNA was prepared from these strains and from *A. terreus* ATCC20542, digested with *EagI*, run out on an agarose gel, and capillary blotted onto a nylon membrane. The membrane was hybridized with the 6.8 kb *BglII* - *KpnI* fragment from pWHM1422 labelled using the Genius II kit using the conditions described previously. The wild type strain had hybridizing bands at 5 kb, 1.5 kb and 1.3 kb.

Mutant strain WMH1734 had hybridizing bands at 4.9 kb, 1.3 kb, 1.0 kb and 0.7 kb confirming the homologous integration of a single copy of the fragment from pWHM1422 at the *lovC* locus. The other mutant strain, WMH1735, had a similar banding pattern but with additional hybridizing bands indicating that multiple integration events had occurred, one of which was at the *lovC* locus.

10 **Construction and characterization of the *A. terreus* strain with extra copies of *lovE*.**

A 10.4 kb *NotI*-*EcoRI* fragment containing the putative regulatory gene, *lovE* was subcloned from pWHM1263 to pSPORT1 to give pWHM1276. From this plasmid a 3.9 kb *HindIII* - *BamHI* fragment was subcloned into pGEM7 to give pWHM1423. The regulatory gene was subcloned from this vector into pPLOA as an *SstI* - *XbaRI* fragment to give pWHM1424 (Fig. 5). pWHM1424 contains nucleotides 30,055 - 33,000 from SEQ ID NO:18 and nucleotides 1 - 1,026 from SEQ ID NO:19.

20 Extra copies of the regulatory gene were introduced into *A. terreus* ATCC20542 by transformation to Zeocin resistance with pWHM1424. Transformants were fermented (method A) and screened for lovastatin production initially by TLC analysis. Most of the transformants appeared to be producing significantly more lovastatin than the wild-type strain. The yields of lovastatin from the two transformant strains, WMH1736 and WMH1737, which had the most elevated levels compared to the wild-type

was quantified by HPLC as described below. These were found to produce 7-fold and 5-fold more lovastatin than the *A. terreus* ATCC20542 strain.

Because of the way that the DNA integrates (ectopically), each transformant is or can be unique, genotypically and phenotypically. However, some will be overproducers; others may exhibit no difference, for unknown reasons.

Heterologous expression of the lovastatin biosynthesis genes.

To place the NPKS gene (*lovB*) under the control of the inducible *alcA* promoter, the 11.5 kb *KpnI* - *AvrII* fragment from pTPKS100 containing the NPKS open reading frame was ligated into pAL3 (Waring, et al., Gene 79:119, 1989) previously digested with *KpnI* and *XbaI*. The resulting plasmid was designated pAL3TPKS (WHM1425). The polymerase chain reaction was used to amplify the NPKS gene sequence between the NPKS promoter region just upstream of the translational start codon and a *AgeI* site internal to NPKS. The design of the forward primer introduced a *KpnI* site 31 bases from the translational start codon allowing the NPKS to be placed against the *alcA* promoter but also incorporating upstream elements from the *A. terreus* system. Amplification was performed using Vent DNA polymerase with pTPKS100 as template and 1 μ mol of each primer in a final volume of 100 μ l using the manufacturer's buffer recommendations. After an initial

denaturation cycle of 10 minutes at 95°C amplification was achieved with 30 cycles of 95°C for 1 minute; 55°C for 1 minute and 72°C for 1.5 minutes. The final cycle was followed by 10 minutes at 72°C to ensure complete
5 polymerization. The amplified product (1.7 kb) was digested with *KpnI* and *AgeI* and ligated into pWHM1425 that had been digested with the same enzymes and gel isolated. The resulting plasmid was designated pAL3TPKSNT (pWHM1426). The region introduced by PCR was
10 sequenced on a ABI automated DNA sequencer to ensure sequence fidelity. This plasmid was then used to transform *A. nidulans* strain A722 (Fungal Genetics Stock Centre) to uridine prototrophy.

Transformants were grown by inoculating 0.5 ml of
15 spore suspension (10^8 c.f.u./ml) in 50 ml YEPD in a 250 ml unbaffled flask. This was then grown for 20 hours at 250 rpm and 37°C (New Brunswick Scientific Series 25 Incubator Shaker). The mycelia were then harvested by filtration through Miracloth (Calbiochem), rinsed with
20 sterile, distilled water, and inoculated into fresh 250 ml unbaffled flasks containing 50 ml AMM + lactose + 10 mM cyclopentanone and grown for a further 20 hours under the same conditions. The mycelia were harvested by
25 filtration using Miracloth (Calbiochem), squeezed as dry as possible and frozen in liquid nitrogen. Protein extracts for SDS-PAGE and western analysis were prepared as described in Kennedy and Turner, Molec. Gen. Genet. (1996), 253:189-197, 1996.

One transformant, WMH1738, was shown to have a large protein (>200 kDa) visible on a SDS-PAGE gel that cross reacted with the affinity purified NPKS antibodies (Panlabs). This strain WMH1738 was transformed to

5 hygromycin B resistance with pWHM1263. Transformant colonies were screened for lovastatin resistance and for the production of new metabolites as described below and two strains WMH1739 and WMH1740 were chosen for further analysis. Both of these strains were found to be

10 significantly resistant (up to 100 μ g/ml on solid media) to lovastatin compared with the host strain. This was analyzed by streaking 10 μ l of a spore suspension on solid AMM plates containing lovastatin at 0, 0.1, 0.5, 1, 5, 10, 50 and 100 μ g/ml and incubating at 37°C. Strains

15 WMH1739 and WMH1740 were compared to strains WMH1741 and WMH1742 which were derivatives of WMH1738 transformed to hygromycin resistance with AN26. Strains WMH1739 and - 1740 exhibited no inhibition of growth at any of these lovastatin concentrations whereas strains WMH1741 and -

20 1742 showed slight inhibition of growth at 5 μ g/ml and almost complete growth inhibition at 50 μ g/ml. The two lovastatin resistant strains were fermented in lovastatin-producing conditions using fermentation method B and extracts were analyzed for lovastatin related

25 metabolites as described below. Both strains were found to produce new metabolites. One compound that was common to both comigrated with monacolin J on TLC and HPLC analysis by the methods described below. Semi-

preparative HPLC was used to partially purify some of this compound, which was then analyzed by HPLC - MS. It had the same mass and fragmentation pattern as authentic monacolin J. The other compound, found in only one of
5 the strains, comigrated with monacolin L on TLC and HPLC.

METHODS

Solid medium for growth of *A. terreus*

For the generation of spore suspensions *A. terreus* strains were grown on CM agar at 30°C for 4 to 5 days.

10 CM Agar (for CM liquid medium the agar was omitted):
50 ml Clutterbuck's salts (Vinci, et al., U.S.

Patent No. 5,744,350)

2 ml Vogel's trace elements (Vinci, et al., U.S.
15 Patent No. 5,744,350)
0.5% Difco Bacto tryptone
0.5% Difco Bacto yeast extract
1% glucose
2% Difco Bacto agar
20 in 1 liter of distilled water

Clutterbuck's salts:

12% NaNO₃
1.02% KCl
1.04% MgSO₄·7H₂O
25 3.04% KH₂PO₄

Vogel's trace elements:

0.004% ZnCl₂
0.02% FeCl₃
0.001% CuCl₂
30 0.001% MnCl₂·4H₂O
0.001% Na₂B₄O₇·10H₂O
0.001% (NH₄)₆Mo₇O₂₄·7H₂O

For long term storage *A. terreus* spores were suspended in SSS (10% glycerol, 5% lactose) and stored at
35 -70°C.

For the generation of spore stocks *A. nidulans* was grown on the following solid growth medium (ACM) for 3 to 4 days at 37°C.

ACM:

- 5 2% Difco Bacto malt extract
- 0.1% Difco Bacto peptone
- 2% glucose
- 2% agar (Difco, Detroit, MI)

For strains which required para-aminobenzoic acid (PABA) for growth, PABA was added to a final concentration of 1 µg/ml. For strains which required uracil and uridine these were added at 20 mM and 10 mM, respectively. Spores were suspended in Tween 80 - saline solution (0.025% Tween 80, 0.8% NaCl) and stored at 4°C.

15 AMM:

- 0.6% (w/v) NaNO₃
- 0.052% (w/v) KCl
- 0.152% (w/v) KH₂PO₄
- 0.052% (w/v) MgSO₄·7H₂O
- 20 1% (w/v) glucose
- 0.1% (v/v) AMM trace elements solution
- pH to 6.5 and make up to 1 liter with distilled water.

For preparation of plates 2% (w/v) Difco Bacto agar was added. If required the glucose can be omitted and an alternative carbon source (e.g., lactose added at the same concentration). For the preparation of transformation plates KCl was added at 4.47% (w/v) (0.6 M).

30 AMM trace elements solution:

- 0.1% (w/v) FeSO₄·7H₂O
- 0.88% (w/v) ZnSO₄·7H₂O
- 0.04% (w/v) CuSO₄·5H₂O
- 0.015% (w/v) MnSO₄·4H₂O
- 35 0.01% (w/v) Na₂B₄O₇·10H₂O

0.005% $(\text{NH}_4)_2\text{Mo}_2\text{O}_7 \cdot 7\text{H}_2\text{O}$
distilled water to 1 liter

Large scale genomic DNA preparation from *A. terreus* for genomic library construction.

5 A 2.5 ml aliquot of spore suspension (10^8 c.f.u./ml) was used to inoculate 500 ml of liquid CM medium and grown for 20 hours at 30°C and 200 rpm. The mycelium was harvested by filtration through Miracloth (Calbiochem) and rinsed extensively with water then TSE [150 mM NaCl,
10 100 mM Na_2EDTA , 50 mM Tris-HCl pH 8.0]. The mycelium was squeezed dry, broken into small pellets and frozen in liquid nitrogen then ground to a fine powder in a pre-chilled pestle and mortar followed by transferral to a 500 ml flask. Fifty ml of extraction buffer [150 mM
15 NaCl, 100 mM Na_2EDTA , 50 mM Tris-HCl pH 8.0, 2% (w/v) SDS] and 10 ml of toluene was added to the flask which was shaken at 60 rpm for 72 hours. This mixture was centrifuged at 1000 x g for 15 minutes and the supernatant was removed and extracted with an equal
20 volume of chloroform:isoamyl alcohol (24:1 vol/vol). This mixture was centrifuged at 10,000 x g for 30 minutes at 15°C. The aqueous layer was carefully removed and 1.1 volumes of ethanol was layered on top. The DNA was spooled out from the resulting suspension and resuspended
25 in 5 ml TE [10 mM Tris-HCl pH 8.0, 1 mM EDTA] + 50 $\mu\text{g/ml}$ RNase and 100 $\mu\text{g/ml}$ proteinase K then incubated at 37°C for 2 hours. The mixture was extracted again with chloroform:isoamyl alcohol (24:1) and the DNA was spooled out as before. Following resuspension in 1 ml of TE the

DNA was extracted once with phenol:chloroform:isoamyl alcohol (25:24:1, vol/vol), once with chloroform:isoamyl alcohol (24:1) and precipitated with 0.6 volumes isopropanol. The DNA clot was removed, dried briefly and
5 resuspended in 0.5 ml TE.

Small scale genomic DNA preparation from *A. terreus* for Southern blot.

A 0.5 ml aliquot of spore suspension (10^8 c.f.u./ml) was used to inoculate 100 ml of liquid CM and grown for
10 20 hours at 30°C and 200 rpm. The mycelium was harvested by filtration through Miracloth (Calbiochem) and rinsed extensively with water then TSE [150 mM NaCl, 100 mM Na₂EDTA, 50 mM Tris-HCl pH 8.0]. The mycelium was squeezed dry, broken into small pellets and frozen in
15 liquid nitrogen. The mycelium was ground to a fine powder in a pre-chilled pestle and mortar and transferred to a mortar pre-heated to 65°C. Three ml of lysis buffer [0.5 M NaCl, 10 mM Tris-HCl pH 7.5, 10 mM EDTA, 1% (w/v) SDS] at 65°C was added and 0.3 ml of 10% (w/v)
20 cetyltrimethylammonium bromide in 0.7 M NaCl. After thorough mixing to form a slurry, 3 ml of phenol:chloroform:isoamyl alcohol (25:24:1) was added. This mixture was transferred to a Corex tube and incubated at 65°C for 15 minutes. Following
25 centrifugation at 12,000 x g for 15 minutes at 4°C the aqueous phase was carefully removed and re-extracted once with phenol, once with phenol:chloroform:isoamyl alcohol (25:24:1) and once with chloroform:isoamyl alcohol (24:1). The DNA was precipitated from the extract by

addition of 0.1 volume of 3 M sodium acetate pH 5 and 0.6 volumes isopropanol then collected by centrifugation (10,000 x g, 10 minutes, 4°C). After washing with 70% ethanol the pellet was briefly dried and resuspended in
5 TE + RNase (50 µg/ml).

Transformation of *A. terreus*.

A 0.5 ml aliquot of spore suspension (10^8 c.f.u./ml) was used to inoculate 100 ml of liquid CM and grown for 20 hours at 30°C and 200 rpm. The mycelium was harvested
10 by centrifugation at 2000 x g for 15 minutes at 4°C and washed twice with an aqueous solution containing 0.27 M CaCl₂ and 0.6 M NaCl. To produce protoplasts the washed mycelia was resuspended in 20 ml of the same solution containing 5 mg/ml Novozym 234 (NovoNordisk) and
15 incubated at 30°C for 1 - 3 hours with gentle agitation. Protoplasts were separated from undigested mycelia by filtration through Miracloth (Calbiochem). The protoplast suspension was diluted with an equal volume of STC1700 [1.2 M sorbitol, 10 mM Tris-HCl pH 7.5, 35 mM
20 NaCl] and incubated on ice for 10 minutes. The protoplasts were collected by centrifugation (2000 x g, 10 minutes, 4°C), washed with STC1700 and resuspended in 1 ml STC1700. Plasmid DNA, purified using Qiagen columns, (2 - 5 µg in 10 µl) was added to 150 µl of
25 protoplast suspension and incubated at room temperature for 25 minutes. PEG solution [60% (w/v) polyethylene glycol 4000, 50 mM CaCl₂, 10 mM Tris-HCl pH 7.5] was added to the DNA/protoplasts mixture in three steps: 250 µl,

250 μ l, and 850 μ l with mixing after each addition. The suspension was incubated at room temperature for 25 minutes then diluted to 10 ml with STC1700. Protoplasts were collected by centrifugation as above and diluted
5 with 500 μ l STC1700. 100 μ l aliquots of this mixture were plated onto osmotically stabilized plates [CM medium containing 3% (w/v) Difco Bacto agar and 23.4% (w/v) mannitol, 15 ml of agar per plate]. After 4 hours growth at 30°C, 25 ml of OL agar [1% (w/v) Difco Bacto peptone,
10 1% (w/v) Difco Bacto agar, 200 μ g/ml Zeocin] was overlaid onto each dish. The plates were incubated for 3 - 4 days at 30°C before transformant colonies were picked. These were streaked to single colonies twice on selective media (CM + 100 μ g/ml Zeocin) before spore
15 suspensions were prepared.

Transformation of *A. nidulans*.

A 0.5 ml aliquot of spore suspension (10^8 c.f.u./ml) was used to inoculate 100 ml of YEPD [2% (w/v) Difco Bacto yeast extract, 2% (w/v) glucose, 0.1% Difco Bacto
20 peptone] liquid medium including necessary supplements and grown for 20 hours at 37°C and 200 rpm. The mycelia was harvested by centrifugation (2000 x g, 10 minutes, 4°C) and washed twice with 0.6 M KCl. To generate protoplasts the mycelia was resuspended in 20 ml of 0.6 M
25 KCl containing 5 mg/ml Novozym 234 and incubated at 30°C for 1 - 2 hours with gentle shaking. Protoplasts were separated from undigested mycelia by filtration through Miracloth (Calbiochem). The protoplasts were harvested

by centrifugation as described above and washed twice with 0.6 M KCl, then resuspended in 10 ml 0.6 M KCl + 50 mM CaCl₂. After counting in a haemocytometer the protoplasts were harvested by centrifugation as before and resuspended to a final concentration of 5×10^8 protoplasts/ml. To 50 μ l of protoplast suspension, 5 μ l of DNA (2 - 5 μ g, purified using Qiagen columns) was added, then 12.5 μ l of PEG solution [25% (w/v) PEG 6000, 50 mM CaCl₂, 10 mM Tris - HCl pH 7.5] and the mixture was incubated on ice for 20 minutes. A further 0.5 ml of PEG solution was added and the mixture was incubated on ice for a further 5 minutes. A 1 ml aliquot of 0.6 M KCl + 50 mM CaCl₂ was added and the protoplasts were plated out in 50 μ l, 200 μ l, and 400 μ l aliquots. For transformation to uridine prototrophy, protoplasts were plated out onto AMM + 0.6 M KCl plates without adding uridine or uracil supplements. Plates were incubated at 37°C for 3 - 4 days when transformants were picked. For transformation to hygromycin B resistance protoplasts were plated out onto AMM + 0.6 M KCl plates (15 ml) and incubated for 4 hours at 30°C. 30 ml of 1% peptone, 1% agar, 1 mg/ml hygromycin B was then used to overlay the plates, which were incubated for 3 - 4 days when transformants were picked. Transformants from both methods were streaked out to single colonies on selective media (i.e., lacking uridine/uracil supplements or containing 1 μ g/ml hygromycin B) twice before spore suspensions were made.

Analysis of strains for lovastatin production.

Two fermentation methods were used for the analysis of lovastatin production. In Method A, 0.5 ml of spore suspension (10^8 c.f.u./ml) was inoculated into 25 ml of SEED medium in 250 ml unbaffled flasks and grown for 18 hours at 250 rpm and 30°C (New Brunswick Scientific Model 25 incubator/shaker). A 1 ml portion of the resulting seed culture was used to inoculate 25 ml of FM in a 250 ml unbaffled flask and grown for 6 days in the conditions described above. Fermentation Method B involved inoculating 50 ml of RPM in a 250 ml unbaffled flask with 0.5 ml of spore suspension (10^8 c.f.u./ml) and growing at 30°C and 250 rpm for 7 days in a New Brunswick Scientific Series 25 Incubator Shaker.

15 SEED medium:

0.5% (w/v) Sigma corn steep liquor
4% (w/v) tomato paste
1% (w/v) oat flour
1% (w/v) glucose
20 1% (v/v) Vogel's trace elements
distilled water to 1 l

FM:

4.5% (w/v) glucose
2.4% (w/v) Sigma peptonized milk
25 0.25% (w/v) Difco Bacto yeast extract
0.25% (w/v) polyethylene glycol 2000
distilled water up to 1 l

RPM:

4% (w/v) lactose
30 0.3% (w/v) rapeseed meal
0.2% (w/v) KNO_3
0.3% (w/v) KH_2PO_4
0.05% (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$
0.05% (w/v) NaCl
35 0.05% (v/v) Sigma antifoam B
0.05% (v/v) trace elements solution
pH to 6.5 and made up to 1 l with distilled water.

Trace elements solution is:

0.16% (w/v) MnSO_4
0.34% (w/v) $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$
0.2% (w/v) $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$
5 0.5% (w/v) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$

made up to 1 liter with distilled water.

The cultures were extracted by adjusting the pH of the media to 3 with HCl, adding an equal volume of ethyl
10 acetate, and shaking the mixture on a New Brunswick Scientific Series 25 incubator/shaker at 250 rpm for 2 hours. For analysis, 1 ml of the ethyl acetate layer was dried under a nitrogen stream and resuspended in 0.1 ml of methanol. For TLC analysis 10 μl of this extract was
15 run on C-18 reverse phase TLC plates (RP-18 F₂₅₄ - Merck) in a solvent system of methanol:0.1% phosphoric acid (9:1). TLC plates were developed by spraying with 10% phosphomolybdic acid in methanol and heating with a heat gun. Extracts were compared with authentic lovastatin,
20 monacolin J, monacolin L, and dihydromonacolin L (acid and lactone forms). For HPLC analysis a Waters Nova-Pak C₁₈ (3.9 x 150 mm) column was used with a solvent system of acetonitrile (B) and 0.1% phosphoric acid (A). The column was eluted with a preprogrammed gradient of 0 to
25 100% B into A over 25 minutes using gradient 7 (Waters Millenium Software) with a flow rate of 1.5 ml/min and metabolites were detected with a Waters 996 Photodiode Array Detector; lovastatin was detected at 238 nm. For purification of metabolites a Waters Prep Nova-Pak HR C₁₈
30 (7.8 x 300 mm) column was used. The same solvent system as above was used with gradient of 0 to 100% B in A over

75 minutes at a flow rate of 4.5 ml/min. Fractions were collected manually, back extracted with ethyl acetate and dried. For HPLC-MS an Aquapore OD-300 7 micron (1.0 x 100 mm) column was used with a gradient of 0 to 100%
5 acetonitrile into A (0.05% TFA) over 30 minutes at a flow rate of 0.02 ml/min.

CLAIMS

We claim:

1. A method of increasing the production of lovastatin in a lovastatin-producing organism, comprising the steps of transforming the organism with the D4B segment, wherein the segment is transcribed and
5 translated, and wherein an increase in lovastatin production occurs.
2. The method of claim 1 wherein the D4B segment is the *A. terreus* D4B segment.
3. The method of claim 1, wherein the D4B segment is identical to nucleotides 579 - 33,000 of SEQ ID NO:18 and 1 - 5,349 of SEQ ID NO:19.
4. The method of claim 1, wherein the lovastatin-producing organism is selected from the group consisting of *A. terreus* ATCC 20542 and ATCC 20541.
5. The method of claim 1, wherein the organism is selected from the group consisting of fungi and yeast.
6. The method of claim 1 wherein the increase is at least 2-fold.

7. The method of claim 1 wherein the nucleic acid sequence is identical to a sequence isolated from ATCC 98876.

8. The method of claim 1 additionally comprising transforming the organism with the entire *A. terreus* lovastatin gene cluster.

9. The method of claim 8 wherein the gene cluster comprises SEQ ID NOs:18 and 19.

10. The method of claim 8 wherein the nucleic acid sequence of the gene cluster is identical to sequences isolated from ATCC 98876 and 98877.

11. A method of increasing the production of monacolin J in a lovastatin-producing organism, comprising the steps of transforming the organism with the D4B segment, wherein the segment is translated, and
5 wherein an increase monacolin J production occurs.

12. A method of increasing the production of lovastatin in a lovastatin-producing organism, comprising the step of transforming the organism with the LovE gene, wherein the nucleic acid sequence is translated, and
5 wherein an increase in lovastatin production occurs.

13. The method of claim 12 wherein the increase is at least 2.0-fold.

14. The method of claim 13 wherein the increase is at least 5-fold.

15. The method of claim 12 wherein the nucleotide sequence of the LovE gene comprises SEQ ID NO:27.

16. A method of increasing the production of lovastatin in a lovastatin-producing organism comprising the steps of transforming the organism with a nucleic acid sequence comprising a truncated version of the A. terreus D4B segment, wherein the nucleic acid sequence is transcribed and translated and wherein an increase in lovastatin production occurs.

17. A method of increasing the production of lovastatin in a lovastatin-producing organism comprising the steps of transforming the organism with a nucleic acid sequence comprising a truncated version of the A. terreus lovastatin-producing gene cluster, wherein the nucleic acid sequence is transcribed and translated and wherein an increase in lovastatin production occurs.

18. A method of increasing or conferring the production of monacolin J in a non-lovastatin-producing organism comprising the steps of transforming the organism with a nucleic acid sequence comprising the D4B
5 segment, wherein the nucleic acid sequence is transcribed and translated and wherein an increase in monacolin J production occurs.

19. The method of claim 18 wherein the D4B segment is the *A. terreus* D4B segment.

20. The method of claim 18 wherein the D4B segment comprises nucleotides 579 - 33,000 of SEQ ID NO:18 and 1-5,349 of SEQ ID NO:19.

21. The method of claim 18 additionally comprising the step of converting the monacolin J into lovastatin.

22. The method of claim 18 additionally comprising the step of transforming the organism with a nucleic acid sequence comprising the LovF gene, wherein the nucleic acid sequence is transcribed and translated and wherein
5 an increase in lovastatin production occurs.

23. An isolated nucleic acid sequence selected from the group consisting of SEQ ID NOs:20 - 36.

24. A lovastatin-producing organism, wherein the organism has been genetically modified to have increased lovastatin production, wherein the increase is at least 2-fold.

25. The organism of claim 24, wherein the organism is a yeast or a fungi.

26. A non-lovastatin producing organism, wherein the organism has been genetically modified to produce monacolin J.

27. The organism of claim 26, wherein the organism is a yeast or a fungi.

28. A non-lovastatin producing organism, wherein the organism has been genetically modified to produce lovastatin.

29. The organism of claim 28 wherein the organism is a yeast or a fungi.

Lovastatin production genes

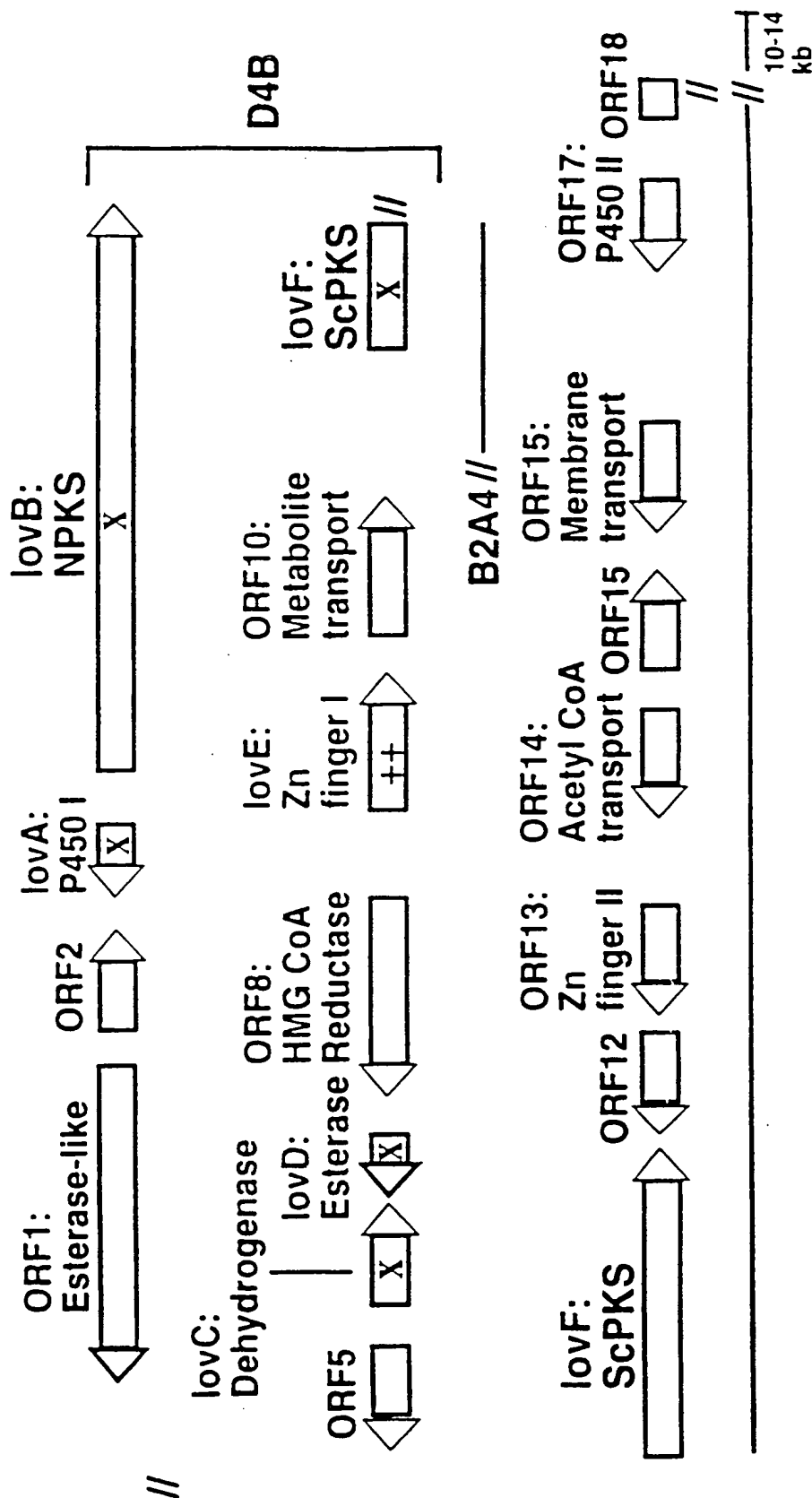


Fig. 1

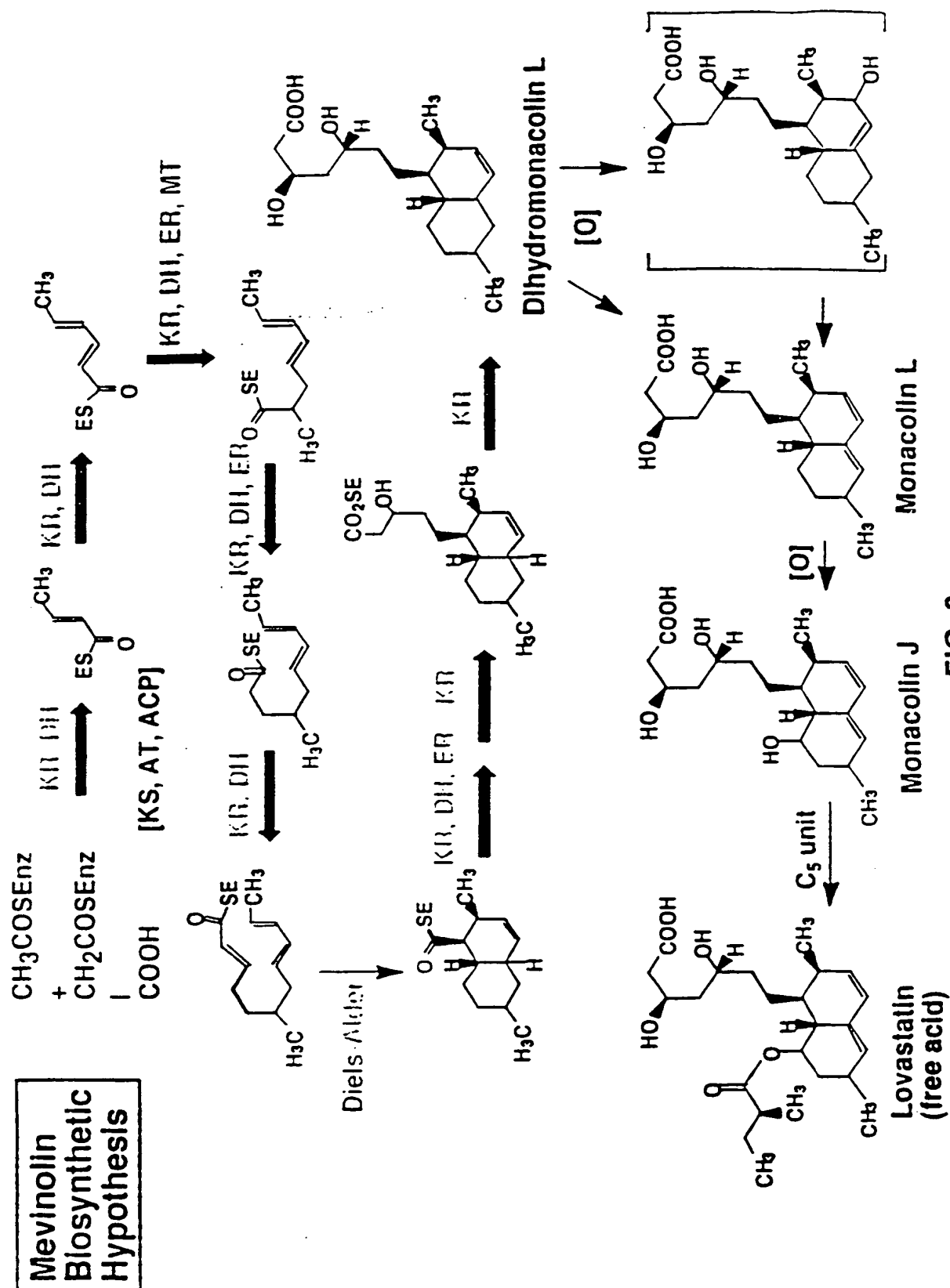
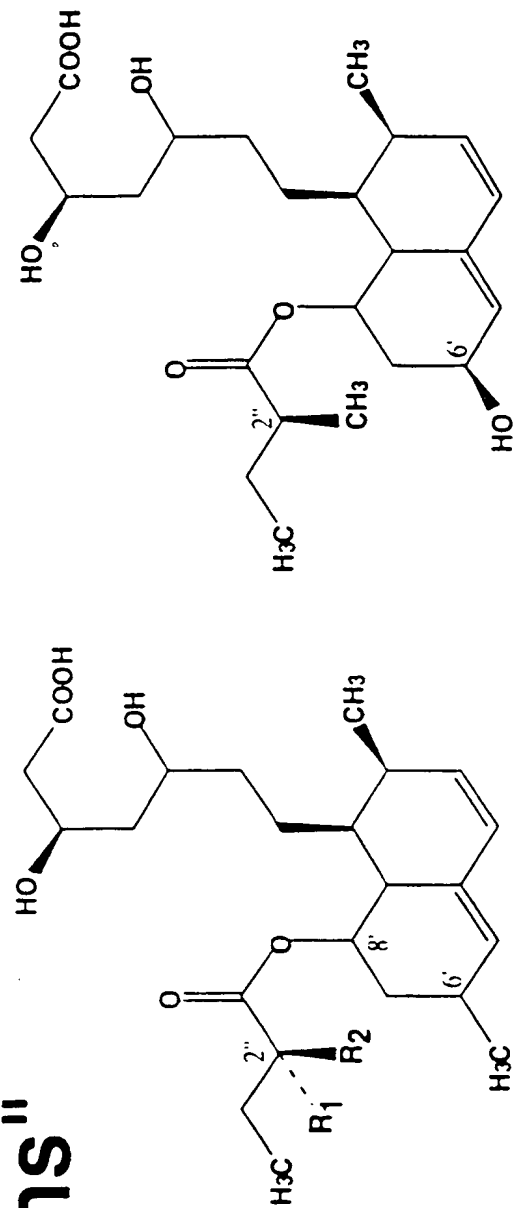


FIG. 2

The "Statins"



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Lovastatin	H	CH ₃
Simvastatin	CH ₃	CH ₃

Pravastatin

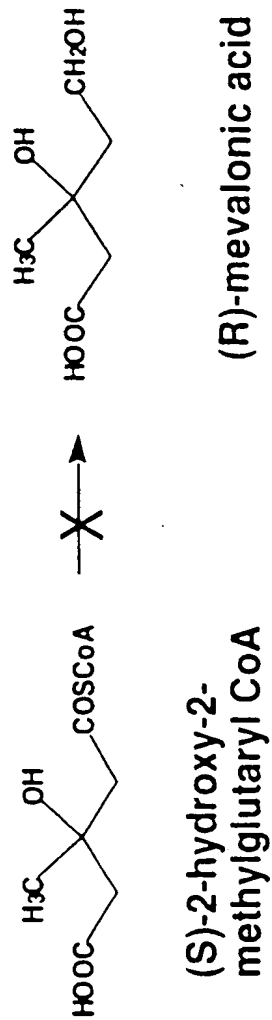


FIG. 3

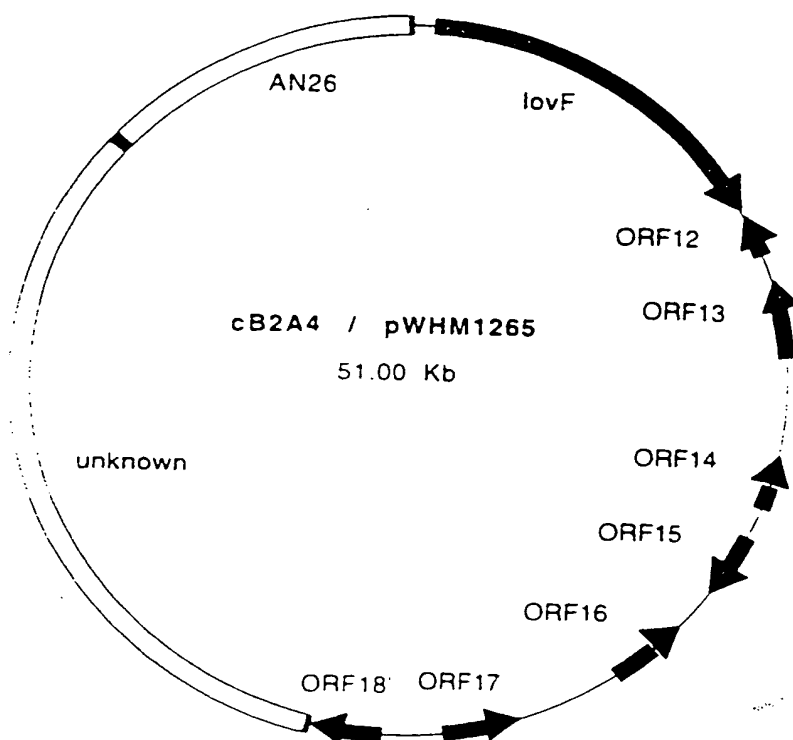


FIG. 4

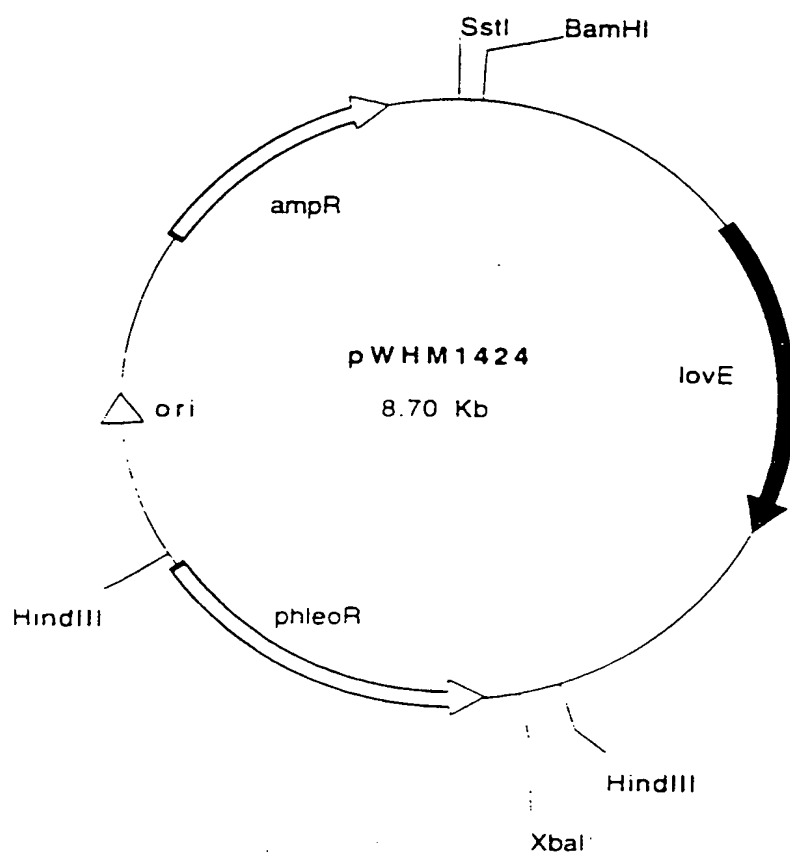


FIG. 5

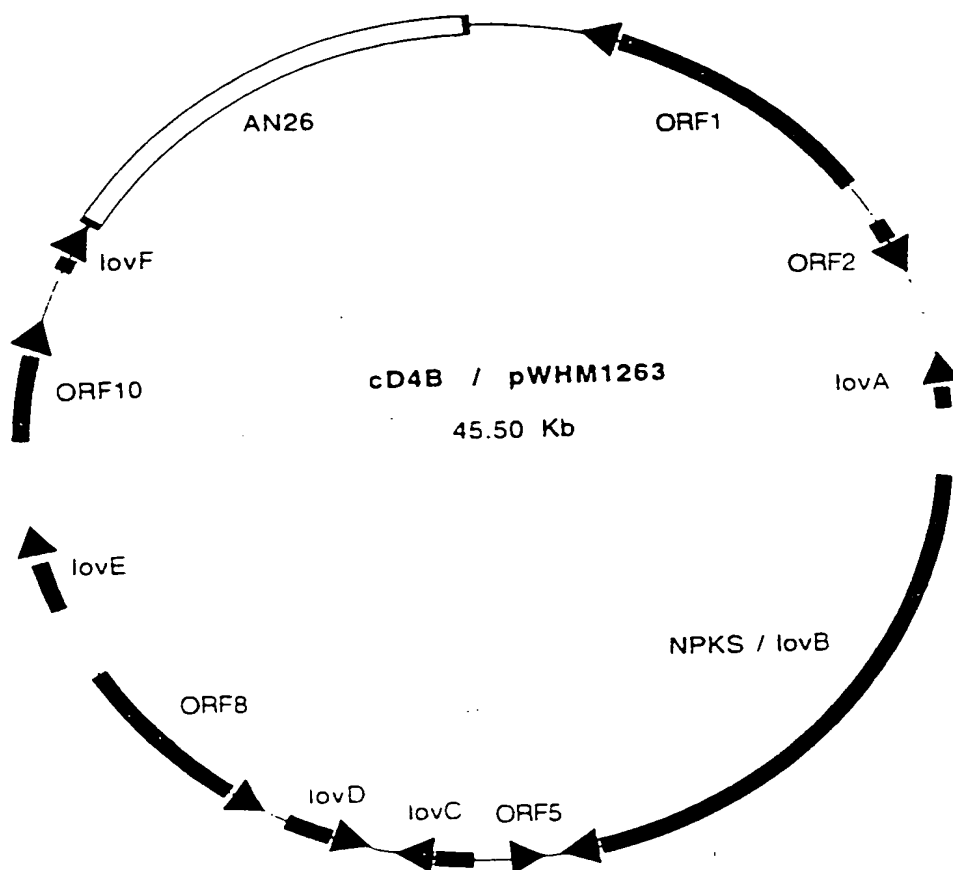


FIG. 6

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Kennedy, Jonathan n.m.i.
Park, Cheonseck n.m.i.

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 Asn Pro Ser Asp Thr Lys Met Arg Gly Gln Phe Ala Thr Pro Trp Ala
 50 55 60
 Phe Leu Gly Thr Asp Tyr Ala Gly Thr Val Val Ala Val Gly Ser Asp
 65 70 75 80
 Val Thr His Ile Gln Val Gly Asp Arg Val Tyr Gly Ala Gln Asn Glu
 85 90 95

Met Cys Pro Arg Thr Pro Asp Gln Gly Ala Phe Ser Gln Tyr Thr Val
 100 105 110

Thr Arg Gly Arg Val Trp Ala Lys Ile Pro Lys Gly Leu Ser Phe Glu
 115 120 125

Gln Ala Ala Ala Leu Pro Ala Gly Ile Ser Thr Ala Gly Leu Ala Met
 130 135 140

Lys Leu Leu Gly Leu Pro Leu Pro Ser Pro Ser Ala Asp Gln Pro Pro
 145 150 155 160

Thr His Ser Lys Pro Val Tyr Val Leu Val Tyr Gly Gly Ser Thr Ala
 165 170 175

Thr Ala Thr Val Thr Met Gln Met Leu Arg Leu Ser Gly Tyr Ile Pro
 180 185 190

Ile Ala Thr Cys Ser Pro His Asn Phe Asp Leu Ala Lys Ser Arg Gly
 195 200 205

Ala Glu Glu Val Phe Asp Tyr Arg Ala Pro Asn Leu Ala Gln Thr Ile
 210 215 220

Arg Thr Tyr Thr Lys Asn Asn Leu Arg Tyr Ala Leu Asp Cys Ile Thr
 225 230 235 240

Asn Val Glu Ser Thr Thr Phe Cys Phe Ala Ala Ile Gly Arg Ala Gly
 245 250 255

Gly His Tyr Val Ser Leu Asn Pro Phe Pro Glu His Ala Ala Thr Arg
 260 265 270

Lys Met Val Thr Thr Asp Trp Thr Leu Gly Pro Thr Ile Phe Gly Glu
 275 280 285

Gly Ser Thr Trp Pro Ala Pro Tyr Gly Arg Pro Gly Ser Glu Glu Glu
 290 295 300

Arg Gln Phe Gly Glu Asp Leu Trp Arg Ile Ala Gly Gln Leu Val Glu
 305 310 315 320

Asp Gly Arg Leu Val His His Pro Leu Arg Val Val Gln Gly Gly Phe
 325 330 335

Asp His Ile Lys Gln Gly Met Glu Leu Val Arg Lys Gly Glu Leu Ser
 340 345 350

Gly Glu Lys Leu Val Val Arg Leu Glu Gly Pro
 355 360

<210> 6

<211> 413

<212> PRT

<213> Aspergillus terreus

<400> 6

Met Gly Ser Ile Ile Asp Ala Ala Ala Ala Ala Asp Pro Val Val Leu
 1 5 10 15

Met Glu Thr Ala Phe Arg Lys Ala Val Lys Ser Arg Gln Ile Pro Gly
 20 25 30

Ala Val Ile Met Ala Arg Asp Cys Ser Gly Asn Leu Asn Tyr Thr Arg
 35 40 45

Cys Phe Gly Ala Arg Thr Val Arg Arg Asp Glu Cys Asn Gly Leu Pro
 50 55 60
 Pro Leu Gln Val Asp Thr Pro Cys Arg Leu Ala Ser Ala Thr Lys Leu
 65 70 75 80
 Leu Thr Thr Ile Met Ala Leu Gln Cys Met Glu Arg Gly Leu Val Asp
 85 90 95
 Leu Asp Glu Thr Val Asp Arg Leu Leu Pro Asp Leu Ser Ala Met Pro
 100 105 110
 Val Leu Glu Gly Phe Asp Asp Ala Gly Asn Ala Arg Leu Arg Glu Arg
 115 120 125
 Arg Gly Lys Ile Thr Leu Arg His Leu Leu Thr His Thr Ser Gly Leu
 130 135 140
 Ser Tyr Val Phe Leu His Pro Leu Leu Arg Glu Tyr Met Ala Gln Gly
 145 150 155 160
 His Leu Gln Ser Ala Glu Lys Phe Gly Ile Glx Ser Arg Leu Ala Pro
 165 170 175
 Pro Ala Val Asn Asp Pro Gly Ala Glu Trp Ile Tyr Gly Ala Asn Leu
 180 185 190
 Asp Trp Ala Gly Lys Leu Val Glu Arg Ala Thr Gly Leu Asp Leu Glu
 195 200 205
 Gln Tyr Leu Gln Glu Asn Ile Cys Ala Pro Leu Gly Ile Thr Asp Met
 210 215 220
 Thr Phe Lys Leu Gln Gln Arg Pro Asp Met Leu Ala Arg Arg Ala Asp
 225 230 235 240
 Gln Thr His Arg Asn Ser Ala Asp Gly Arg Leu Arg Tyr Asp Asp Ser
 245 250 255
 Val Tyr Phe Arg Ala Asp Gly Glu Glu Cys Phe Gly Gly Gln Gly Val
 260 265 270
 Phe Ser Gly Pro Gly Ser Tyr Met Lys Val Leu His Ser Leu Leu Lys
 275 280 285
 Arg Asp Gly Leu Leu Leu Gln Pro Gln Thr Val Asp Leu Met Phe Gln
 290 295 300
 Pro Ala Leu Glu Pro Arg Leu Glu Glu Gln Met Asn Gln His Met Asp
 305 310 315 320
 Ala Ser Pro His Ile Asn Tyr Gly Gly Pro Met Pro Met Val Leu Arg
 325 330 335
 Arg Ser Phe Gly Leu Gly Gly Ile Ile Ala Leu Glu Asp Leu Asp Gly
 340 345 350
 Glu Asn Trp Arg Arg Lys Gly Ser Leu Thr Phe Gly Gly Gly Pro Asn
 355 360 365
 Ile Val Trp Gln Ile Asp Pro Lys Ala Gly Leu Cys Thr Leu Ala Phe
 370 375 380
 Phe Gln Leu Glu Pro Trp Asn Asp Pro Val Cys Arg Asp Leu Thr Arg
 385 390 395 400

Thr Phe Glu His Ala Ile Tyr Ala Gln Tyr Gln Gln Gly
405 410

<210> 7
<211> 1068
<212> PRT
<213> *Aspergillus terreus*

<400> 7

Met Asp Pro Val Val Arg Lys Pro Asp Pro Gly Gly Val Gln His Arg
1 5 10 15
Val Thr Lys Ala Leu Arg Ala Ile Val Gly His Ala Cys Arg His Pro
20 25 30
Ile His Thr Leu Leu Val Thr Ala Leu Thr Ala Ala Thr Thr His Leu
35 40 45
His Val Leu Glu Gly Thr Tyr Gln Ala Thr His Arg Glu Ala Ser Ala
50 55 60
Trp Lys Trp Gln Ile Asp Asp Arg Pro Lys Val Pro Glu Asp Gly Gln
65 70 75 80
Ser Asp Phe His Trp Ala Leu Val Thr Leu Asp Leu Pro Gly Ala Ser
85 90 95
Val Asp Ala Ser Ile Pro Phe Leu Ser Asn Thr Leu Ser Gly Phe Leu
100 105 110
Gly Ala Glu Gln Thr Thr Pro Thr Pro Asp Ser Ser Pro Ser Pro Asp
115 120 125
His Ser Ala Leu Thr Phe Arg Val Pro Tyr Ser Gln Leu Asp Gly Phe
130 135 140
Leu Gln Ala Val Glu Ile Ile Pro Ser Glu Lys Glu Asp Asp Ser Trp
145 150 155 160
Arg Leu Arg Ser Pro Arg Glu Glu Gly Ser Pro Arg Ser Leu Gly His
165 170 175
Trp Leu Gly Ser Ser Trp Leu Ser Phe Leu His Arg Val His His Ala
180 185 190
Glu Thr Val Asp Leu Val Ile Ile Gly Leu Ser Tyr Leu Ala Met Asn
195 200 205
Met Thr Val Val Ser Leu Phe Arg Val Met Arg His Leu Gly Ser Arg
210 215 220
Phe Trp Leu Ala Ala Ser Val Leu Leu Ser Gly Ala Phe Ala Phe Val
225 230 235 240
Leu Gly Leu Gly Ile Thr Thr Thr Cys Asp Val Pro Val Asp Met Leu
245 250 255
Leu Leu Phe Glu Gly Ile Pro Tyr Leu Val Leu Thr Val Gly Phe Glu
260 265 270
Lys Pro Ile Gln Leu Thr Arg Ala Val Leu Cys Val Ser Glu Glu Leu
275 280 285
Trp Gly Gly Gly Gln Arg Gln Val Pro Asn Gly Ala Ser Ser Asp Asp
290 295 300

Ser Arg Gln Asn Gln Leu Ile Pro Asn Ile Ile Gln Leu Ala Val Asp
 305 310 315 320
 Arg Glu Gly Trp Tyr Ile Val Arg Ser Tyr Leu Leu Glu Ile Gly Ala
 325 330 335
 Leu Ala Leu Gly Ala Val Leu Arg Pro Lys Asp Ser Leu Gly His Phe
 340 345 350
 Cys Phe Leu Ala Ala Trp Thr Leu Leu Ile Asp Ala Val Leu Leu Phe
 355 360 365
 Thr Phe Tyr Ala Thr Ile Leu Cys Val Lys Leu Glu Ile Thr Arg Ile
 370 375 380
 Arg Ser Pro Gly Gly Leu Gly Gln Val Asn Ala Lys His Pro Ser Gly
 385 390 395 400
 Ile Phe Gly His Lys Val Lys Ser Thr Asn Ile Thr Trp Trp Lys Leu
 405 410 415
 Leu Thr Val Gly Gly Phe Val Leu Cys His Phe Leu Gln Leu Ser Pro
 420 425 430
 Phe Phe Tyr Arg Val Met Gly Glu Tyr Met Ala Asn Gly Thr Leu Pro
 435 440 445
 Pro Thr Ala Val Ser Pro Phe Lys Glu Ala Ala Asn Gly Leu Asn Glu
 450 455 460
 Ile Tyr Leu Thr Ala Arg Val Glu Gly Phe Glu Thr Arg Val Thr Val
 465 470 475 480
 Leu Pro Pro Leu Gln Tyr Val Leu Glu Ser Ala Gly Phe Asn Ile Ser
 485 490 495
 Ala Thr Lys Arg Ser Thr Phe Asp Gly Val Leu Asp Gly Leu Glu Ser
 500 505 510
 Pro Leu Gly Arg Leu Cys Leu Met Gly Ala Leu Val Val Ser Leu Val
 515 520 525
 Leu Asn Asn His Leu Ile His Ala Ala Arg Trp His Ala Trp Pro Gln
 530 535 540
 Ala Arg Glu Ser Ala Val Pro Asp Gly Ser Tyr Leu Ser Val Pro Cys
 545 550 555 560
 Ser Ala Thr Ala Pro Glu Val Cys Thr Arg Pro Pro Glu Glu Thr Glu
 565 570 575
 Ala Leu Leu Lys Ser Asn Gln Ala Glu Ser Leu Thr Asp Asp Glu Leu
 580 585 590
 Val Glu Leu Cys Leu Arg Gly Lys Ile Ala Gly Tyr Ser Leu Glu Lys
 595 600 605
 Thr Leu Glu Arg Ile Ala Ala Gly Ser Ser Arg Ser Val Thr Arg Leu
 610 615 620
 Glu Ala Phe Thr Arg Ala Val Arg Ile Arg Arg Ala Ala Val Ser Lys
 625 630 635 640
 Thr Pro Ser Thr Gln Asn Leu Cys Ser Gly Leu Ala Glu Ser Leu Leu
 645 650 655

Pro Tyr Arg Asp Tyr Asn Tyr Glu Leu Val His Gly Ala Cys Cys Glu
 660 665 670
 Asn Val Val Gly Tyr Leu Pro Leu Pro Leu Gly Val Ala Gly Pro Met
 675 680 685
 Val Ile Asp Gly Gln Ala Leu Phe Ile Pro Met Ala Thr Thr Glu Gly
 690 695 700
 Val Leu Val Ala Ser Ala Ser Arg Gly Cys Lys Ala Ile Asn Ala Gly
 705 710 715 720
 Gly Gly Ala Thr Thr Met Leu Lys Gly Asp Gly Met Thr Arg Gly Pro
 725 730 735
 Cys Leu Arg Phe Pro Ser Ala Gln Arg Ala Ala Glu Ala Gln Arg Trp
 740 745 750
 Val Glu Ser Pro Leu Gly His Glu Val Leu Ala Ala Ala Phe Asn Ala
 755 760 765
 Thr Ser Arg Phe Ala Arg Leu Gln Thr Leu Thr Val Ala Gln Ala Gly
 770 775 780
 Ile Tyr Leu Tyr Ile Arg Phe Arg Thr Thr Thr Gly Asp Ala Met Gly
 785 790 795 800
 Met Asn Met Ile Ser Lys Gly Val Glu Lys Ala Leu Glu Ala Met Ala
 805 810 815
 Ala Glu Gly Gly Phe Pro Asp Met His Thr Val Thr Leu Ser Gly Asn
 820 825 830
 Phe Cys Ser Asp Lys Lys Ser Ala Ala Ile Asn Trp Ile Gly Gly Arg
 835 840 845
 Gly Lys Ser Val Ile Ala Glu Ala Thr Ile Pro Ala Glu Thr Val Arg
 850 855 860
 Gln Val Leu Lys Thr Asp Val Asp Ala Leu Val Glu Leu Asn Thr Ala
 865 870 875 880
 Lys Asn Leu Val Gly Ser Ala Met Ala Gly Ser Leu Gly Gly Phe Asn
 885 890 895
 Ala His Ala Ser Asn Leu Val Gln Ala Val Phe Leu Ala Thr Gly Gln
 900 905 910
 Asp Pro Ala Gln Asn Val Glu Ser Ser Ser Cys Ile Thr Thr Met Lys
 915 920 925
 Asn Ile Asp Gly Asn Leu His Ile Ala Val Ser Met Pro Ser Met Glu
 930 935 940
 Val Gly Thr Ile Gly Gly Gly Thr Ile Leu Glu Ala Gln Gly Ala Met
 945 950 955 960
 Leu Asp Leu Leu Gly Val Arg Gly Ala His Ser Thr Glu Pro Gly Ala
 965 970 975
 Asn Ala Arg Arg Leu Ala Arg Ile Val Ala Ala Ala Val Leu Ala Gly
 980 985 990
 Glu Leu Ser Thr Cys Ala Ala Leu Ala Ala Gly His Leu Val Asn Ala
 995 1000 1005

His Met Gln His Asn Arg Thr Ser Lys Asp Ala Ile Ser Gly Thr Glu
1010 1015 1020

Tyr Gly Ala Ile Arg Thr Pro Val Tyr Val Val Ile Leu Glu His Ala
1025 1030 1035 1040

Gly Asp Ile His Phe Val Gln Ile Glu Tyr Lys Asn Thr Tyr Leu Arg
1045 1050 1055

Arg Lys Val Pro Thr Leu Ser Cys Asn Leu Gly Arg
1060 1065

<210> 8
<211> 503
<212> PRT
<213> *Aspergillus terreus*

<400> 8
Met Ala Ala Asp Gln Gly Ile Phe Thr Asn Ser Val Thr Leu Ser Pro
1 5 10 15

Val Glu Gly Ser Arg Thr Gly Gly Thr Leu Pro Arg Arg Ala Phe Arg
20 25 30

Arg Ser Cys Asp Arg Cys His Ala Gln Lys Ile Lys Cys Thr Gly Asn
35 40 45

Lys Glu Val Thr Gly Arg Ala Pro Cys Gln Arg Cys Gln Gln Ala Gly
50 55 60

Leu Arg Cys Val Tyr Ser Glu Arg Cys Pro Lys Arg Lys Leu Arg Gln
65 70 75 80

Ser Arg Ala Ala Asp Leu Val Ser Ala Asp Pro Asp Pro Cys Leu His
85 90 95

Met Ser Ser Pro Pro Val Pro Ser Gln Ser Leu Pro Leu Asp Val Ser
100 105 110

Glu Ser His Ser Ser Asn Thr Ser Arg Gln Phe Leu Asp Pro Pro Asp
115 120 125

Ser Tyr Asp Trp Ser Trp Thr Ser Ile Gly Thr Asp Glu Ala Ile Asp
130 135 140

Thr Asp Cys Trp Gly Leu Ser Gln Cys Asp Gly Gly Phe Ser Cys Gln
145 150 155 160

Leu Glu Pro Thr Leu Pro Asp Leu Pro Ser Pro Phe Glu Ser Thr Val
165 170 175

Glu Lys Ala Pro Leu Pro Pro Val Ser Ser Asp Ile Ala Arg Ala Ala
180 185 190

Ser Ala Gln Arg Glu Leu Phe Asp Asp Leu Ser Ala Val Ser Gln Glu
195 200 205

Leu Glu Glu Ile Leu Leu Ala Val Thr Val Glu Trp Pro Lys Gln Glu
210 215 220

Ile Trp Thr Arg Ala Ser Pro His Ser Pro Thr Ala Ser Arg Glu Arg
225 230 235 240

Ile Ala Gln Arg Arg Gln Asn Val Trp Ala Asn Trp Leu Thr Asp Leu
245 250 255

His Met Phe Ser Leu Asp Pro Ile Gly Met Phe Phe Asn Ala Ser Arg
 260 265 270
 Arg Leu Leu Thr Val Leu Arg Gln Gln Ala Gln Ala Asp Cys His Gln
 275 280 285
 Gly Thr Leu Asp Glu Cys Leu Arg Thr Lys Asn Leu Phe Thr Ala Val
 290 295 300
 His Cys Tyr Ile Leu Asn Val Arg Ile Leu Thr Ala Ile Ser Glu Leu
 305 310 315 320
 Leu Leu Ser Gln Ile Arg Arg Thr Gln Asn Ser His Met Ser Pro Leu
 325 330 335
 Glu Gly Ser Arg Ser Gln Ser Pro Ser Arg Asp Asp Thr Ser Ser Ser
 340 345 350
 Ser Gly His Ser Ser Val Asp Thr Ile Pro Phe Phe Ser Glu Asn Leu
 355 360 365
 Pro Ile Gly Glu Leu Phe Ser Tyr Val Asp Pro Leu Thr His Ala Leu
 370 375 380
 Phe Ser Ala Cys Thr Thr Leu His Val Gly Val Gln Leu Leu Arg Glu
 385 390 395 400
 Asn Glu Ile Thr Leu Gly Val His Ser Ala Gln Gly Ile Ala Ala Ser
 405 410 415
 Ile Ser Met Ser Gly Glu Pro Gly Glu Asp Ile Ala Arg Thr Gly Ala
 420 425 430
 Thr Asn Ser Ala Arg Cys Glu Glu Gln Pro Thr Thr Pro Ala Ala Arg
 435 440 445
 Val Leu Phe Met Phe Leu Ser Asp Glu Gly Ala Phe Gln Glu Ala Lys
 450 455 460
 Ser Ala Gly Ser Arg Gly Arg Thr Ile Ala Ala Leu Arg Arg Cys Tyr
 465 470 475 480
 Glu Asp Ile Phe Ser Leu Ala Arg Lys His Lys His Gly Met Leu Arg
 485 490 495
 Asp Leu Asn Asn Ile Pro Pro
 500

<210> 9
 <211> 542
 <212> PRT
 <213> Aspergillus terreus

<400> 9
 Met Thr Ser His His Gly Glu Thr Glu Lys Pro Gln Ser Asn Thr Ala
 1 5 10 15
 Gln Met Gln Ile Asn His Val Thr Gly Leu Arg Leu Gly Leu Val Val
 20 25 30
 Val Ser Val Thr Leu Val Ala Phe Leu Met Leu Leu Asp Met Ser Ile
 35 40 45
 Ile Val Thr Ala Ile Pro His Ile Thr Ala Gln Phe His Ser Leu Gly
 50 55 60

Asp Val Gly Trp Tyr Gly Ser Ala Tyr Leu Leu Ser Ser Cys Ala Leu
 65 70 75 80
 Gln Pro Leu Ala Gly Lys Leu Tyr Thr Leu Thr Leu Lys Tyr Thr
 85 90 95
 Phe Leu Ala Phe Leu Gly Leu Phe Glu Ile Gly Ser Val Leu Cys Gly
 100 105 110
 Thr Ala Arg Ser Ser Thr Met Leu Ile Val Gly Arg Ala Val Ala Gly
 115 120 125
 Met Gly Gly Ser Gly Leu Thr Asn Gly Ala Ile Thr Ile Leu Ser Ala
 130 135 140
 Ala Ala Pro Lys Gln Gln Gln Pro Leu Leu Ile Gly Ile Met Met Gly
 145 150 155 160
 Leu Ser Gln Ile Ala Ile Val Cys Gly Pro Leu Leu Gly Gly Ala Phe
 165 170 175
 Thr Gln His Ala Ser Trp Arg Trp Cys Phe Tyr Ile Asn Leu Pro Ile
 180 185 190
 Gly Ala Phe Ala Thr Phe Leu Leu Leu Val Ile Gln Ile Pro Asn Arg
 195 200 205
 Leu Pro Ser Thr Ser Asp Ser Thr Thr Asp Gly Thr Asn Pro Lys Arg
 210 215 220
 Arg Gly Ala Arg Asp Val Leu Thr Gln Leu Asp Phe Leu Gly Phe Val
 225 230 235 240
 Leu Phe Ala Gly Phe Ala Ile Met Ile Ser Leu Ala Leu Glu Trp Gly
 245 250 255
 Gly Ser Asp Tyr Ala Trp Asn Ser Ser Val Ile Ile Gly Leu Phe Cys
 260 265 270
 Ala Ala Gly Val Ser Leu Val Leu Phe Gly Cys Trp Glu Arg His Val
 275 280 285
 Gly Gly Ala Val Ala Met Ile Pro Ile Ser Val Ala Ser Arg Arg Gln
 290 295 300
 Val Trp Cys Ser Cys Phe Phe Leu Gly Phe Phe Ser Gly Ala Leu Leu
 305 310 315 320
 Ile Phe Ser Tyr Tyr Leu Pro Ile Tyr Phe Gln Ala Val Lys Asn Val
 325 330 335
 Ser Pro Thr Met Ser Gly Val Tyr Met Leu Pro Gly Ile Gly Gly Gln
 340 345 350
 Ile Val Met Ala Ile Val Thr Gly Ala Ile Ile Gly Lys Thr Gly Tyr
 355 360 365
 Tyr Val Pro Trp Ala Leu Ala Ser Gly Ile Leu Val Ser Ile Ser Ala
 370 375 380
 Gly Leu Val Ser Thr Phe Gln Pro Glu Thr Ser Ile Ala Ala Trp Val
 385 390 395 400
 Met Tyr Gln Phe Leu Gly Gly Val Gly Arg Gly Cys Gly Met Gln Thr
 405 410 415

Pro Val Val Ala Ile Gln Asn Ala Leu Pro Pro Gln Thr Ser Pro Ile
 420 425 430
 Gly Ile Ser Leu Ala Met Phe Gly Gln Thr Phe Gly Gly Ser Leu Phe
 435 440 445
 Leu Thr Leu Thr Glu Leu Val Phe Ser Asn Gly Leu Asp Ser Gly Leu
 450 455 460
 Arg Gln Tyr Ala Pro Thr Leu Asn Ala Gln Glu Val Thr Ala Ala Gly
 465 470 475 480
 Ala Thr Gly Phe Arg Gln Val Val Pro Ala Pro Leu Ile Ser Arg Val
 485 490 495
 Leu Leu Ala Tyr Ser Lys Gly Val Asp His Ala Phe Tyr Val Ala Val
 500 505 510
 Gly Ala Ser Gly Ala Thr Phe Ile Phe Ala Trp Gly Met Gly Arg Leu
 515 520 525
 Ala Trp Arg Gly Trp Arg Met Gln Glu Lys Gly Arg Ser Glu
 530 535 540

<210> 10
 <211> 2532
 <212> PRT
 <213> *Aspergillus terreus*

<400> 10
 Met Thr Pro Leu Asp Ala Pro Gly Ala Pro Ala Pro Ile Ala Met Val
 1 5 10 15
 Gly Met Gly Cys Arg Phe Gly Gly Gly Ala Thr Asp Pro Gln Lys Leu
 20 25 30
 Trp Lys Leu Leu Glu Glu Gly Gly Ser Ala Trp Ser Lys Ile Pro Pro
 35 40 45
 Ser Arg Phe Asn Val Gly Gly Val Tyr His Pro Asn Gly Gln Arg Val
 50 55 60
 Gly Ser Met His Val Arg Gly Gly His Phe Leu Asp Glu Asp Pro Ala
 65 70 75 80
 Leu Phe Asp Ala Ser Phe Phe Asn Met Ser Thr Glu Val Ala Ser Cys
 85 90 95
 Met Asp Pro Gln Tyr Arg Leu Ile Leu Glu Val Val Tyr Glu Ala Leu
 100 105 110
 Glu Ala Ala Gly Ile Pro Leu Glu Gln Val Ser Gly Ser Lys Thr Gly
 115 120 125
 Val Phe Ala Gly Thr Met Tyr His Asp Tyr Gln Gly Ser Phe Gln Arg
 130 135 140
 Gln Pro Glu Ala Leu Pro Arg Tyr Phe Ile Thr Gly Asn Ala Gly Thr
 145 150 155 160
 Met Leu Ala Asn Arg Val Ser His Phe Tyr Asp Leu Arg Gly Pro Ser
 165 170 175
 Val Ser Ile Asp Thr Ala Cys Ser Thr Thr Leu Thr Ala Leu His Leu
 180 185 190

Ala Ile Gln Ser Leu Arg Ala Gly Glu Ser Asp Met Ala Ile Val Ala
 195 200 205
 Gly Ala Asn Leu Leu Leu Asn Pro Asp Val Phe Thr Thr Met Ser Asn
 210 215 220
 Leu Gly Phe Leu Ser Ser Asp Gly Ile Ser Tyr Ser Phe Asp Ser Arg
 225 230 235 240
 Ala Asp Gly Tyr Gly Arg Gly Glu Gly Val Ala Ala Ile Val Leu Lys
 245 250 255
 Thr Leu Pro Asp Ala Val Arg Asp Gly Asp Pro Ile Arg Leu Ile Val
 260 265 270
 Arg Glu Thr Ala Ile Asn Gln Asp Gly Arg Thr Pro Ala Ile Ser Thr
 275 280 285
 Pro Ser Gly Glu Ala Gln Glu Cys Leu Ile Gln Asp Cys Tyr Gln Lys
 290 295 300
 Ala Gln Leu Asp Pro Lys Gln Thr Ser Tyr Val Glu Ala His Gly Thr
 305 310 315 320
 Gly Thr Arg Ala Gly Asp Pro Leu Glu Leu Ala Val Ile Ser Ala Ala
 325 330 335
 Phe Pro Gly Gln Gln Ile Gln Val Gly Ser Val Lys Ala Asn Ile Gly
 340 345 350
 His Thr Glu Ala Val Ser Gly Leu Ala Ser Leu Ile Lys Val Ala Leu
 355 360 365
 Ala Val Glu Lys Gly Val Ile Pro Pro Asn Ala Arg Phe Leu Gln Pro
 370 375 380
 Ser Lys Lys Leu Leu Lys Asp Thr His Ile Gln Ile Pro Leu Cys Ser
 385 390 395 400
 Gln Ser Trp Ile Pro Thr Asp Gly Val Arg Arg Ala Ser Ile Asn Asn
 405 410 415
 Phe Gly Phe Gly Gly Ala Asn Ala His Ala Ile Val Glu Gln Tyr Gly
 420 425 430
 Pro Phe Ala Glu Thr Ser Ile Cys Pro Pro Asn Gly Tyr Ser Gly Asn
 435 440 445
 Tyr Asp Gly Asn Leu Gly Thr Asp Gln Ala His Ile Tyr Val Leu Ser
 450 455 460
 Ala Lys Asp Glu Asn Ser Cys Met Arg Met Val Ser Arg Leu Cys Asp
 465 470 475 480
 Tyr Ala Thr His Ala Arg Pro Ala Asp Asp Leu Gln Leu Leu Ala Asn
 485 490 495
 Ile Ala Tyr Thr Leu Gly Ser Arg Arg Ser Asn Phe Arg Trp Lys Ala
 500 505 510
 Val Cys Thr Ala His Ser Leu Thr Gly Leu Ala Gln Asn Leu Ala Gly
 515 520 525
 Glu Gly Met Arg Pro Ser Lys Ser Ala Asp Gln Val Arg Leu Gly Trp
 530 535 540

Val Phe Thr Gly Gln Gly Ala Gln Trp Phe Ala Met Gly Arg Glu Leu
 545 550 555 560
 Ile Glu Met Tyr Pro Val Phe Lys Glu Ala Leu Leu Glu Cys Asp Gly
 565 570 575
 Tyr Ile Lys Glu Met Gly Ser Thr Trp Ser Ile Ile Glu Glu Leu Ser
 580 585 590
 Arg Pro Glu Thr Glu Ser Arg Val Asp Gln Ala Glu Phe Ser Leu Pro
 595 600 605
 Leu Ser Thr Ala Leu Gln Ile Ala Leu Val Arg Leu Leu Trp Ser Trp
 610 615 620
 Asn Ile Gln Pro Val Ala Val Thr Ser His Ser Ser Gly Glu Ala Ala
 625 630 635 640
 Ala Ala Tyr Ala Ile Gly Ala Leu Thr Ala Arg Ser Ala Ile Gly Ile
 645 650 655
 Ser Tyr Ile Arg Gly Ala Leu Thr Ala Arg Asp Arg Leu Ala Ser Val
 660 665 670
 His Lys Gly Gly Met Leu Ala Val Gly Leu Ser Arg Ser Glu Val Gly
 675 680 685
 Ile Tyr Ile Arg Gln Val Pro Leu Gln Ser Glu Glu Cys Leu Val Val
 690 695 700
 Gly Cys Val Asn Ser Pro Ser Ser Val Thr Val Ser Gly Asp Leu Ser
 705 710 715 720
 Ala Ile Ala Lys Leu Glu Glu Leu Leu His Ala Asp Arg Ile Phe Ala
 725 730 735
 Arg Arg Leu Lys Val Thr Gln Ala Phe His Ser Ser His Met Asn Ser
 740 745 750
 Met Thr Asp Ala Phe Arg Ala Gly Leu Thr Glu Leu Phe Gly Ala Asp
 755 760 765
 Pro Ser Asp Ala Ala Asn Ala Ser Lys Asp Val Ile Tyr Ala Ser Pro
 770 775 780
 Arg Thr Gly Ala Arg Leu His Asp Met Asn Arg Leu Arg Asp Pro Ile
 785 790 795 800
 His Trp Val Glu Cys Met Leu His Pro Val Glu Phe Glu Ser Ala Phe
 805 810 815
 Arg Arg Met Cys Leu Asp Glu Asn Asp His Met Pro Lys Val Asp Arg
 820 825 830
 Val Ile Glu Ile Gly Pro His Gly Ala Leu Gly Gly Pro Ile Lys Gln
 835 840 845
 Ile Met Gln Leu Pro Glu Leu Ala Thr Cys Asp Ile Pro Tyr Leu Ser
 850 855 860
 Cys Leu Ser Arg Gly Lys Ser Ser Leu Ser Thr Leu Arg Leu Leu Ala
 865 870 875 880
 Ser Glu Leu Ile Arg Ala Gly Phe Pro Val Asp Leu Asn Ala Ile Asn
 885 890 895

Phe Pro Arg Gly Cys Glu Ala Ala Arg Val Gln Val Leu Ser Asp Leu
 900 905 910
 Pro Pro Tyr Pro Trp Asn His Glu Thr Arg Tyr Trp Lys Glu Pro Arg
 915 920 925
 Ile Ser Gln Ser Ala Arg Gln Arg Lys Gly Pro Val His Asp Leu Ile
 930 935 940
 Gly Leu Gln Glu Pro Leu Asn Leu Pro Leu Ala Arg Ser Trp His Asn
 945 950 955 960
 Val Leu Arg Val Ser Asp Leu Pro Trp Leu Arg Asp His Val Val Gly
 965 970 975
 Ser His Ile Val Phe Pro Gly Ala Gly Phe Val Cys Met Ala Val Met
 980 985 990
 Gly Ile Ser Thr Leu Cys Ser Ser Asp His Glu Ser Asp Asp Ile Ser
 995 1000 1005
 Tyr Ile Leu Arg Asp Val Asn Phe Ala Gln Ala Leu Ile Leu Pro Ala
 1010 1015 1020
 Asp Gly Glu Glu Gly Ile Asp Leu Arg Leu Thr Ile Cys Ala Pro Asp
 1025 1030 1035 1040
 Gln Ser Leu Gly Ser Gln Asp Trp Gln Arg Phe Leu Val His Ser Ile
 1045 1050 1055
 Thr Ala Asp Lys Asn Asp Trp Thr Glu His Cys Thr Gly Leu Val Arg
 1060 1065 1070
 Ala Glu Met Asp Gln Pro Pro Ser Leu Ser Asn Gln Gln Arg Ile
 1075 1080 1085
 Asp Pro Arg Pro Trp Ser Arg Lys Thr Ala Pro Gln Glu Leu Trp Asp
 1090 1095 1100
 Ser Leu His Arg Val Gly Ile Arg His Gly Pro Phe Phe Arg Asn Ile
 1105 1110 1115 1120
 Thr Cys Ile Glu Ser Asp Gly Arg Gly Ser Trp Cys Thr Phe Ala Ile
 1125 1130 1135
 Ala Asp Thr Ala Ser Ala Met Pro His Ala Tyr Glu Ser Gln His Ile
 1140 1145 1150
 Val His Pro Thr Thr Leu Asp Ser Ala Val Gln Ala Ala Tyr Thr Thr
 1155 1160 1165
 Leu Pro Phe Ala Gly Ser Arg Ile Lys Ser Ala Met Val Pro Ala Arg
 1170 1175 1180
 Val Gly Cys Met Lys Ile Ser Ser Arg Leu Ala Asp Leu Glu Ala Arg
 1185 1190 1195 1200
 Asp Met Leu Arg Ala Gln Ala Lys Met His Ser Gln Ser Pro Ser Ala
 1205 1210 1215
 Leu Val Thr Asp Val Ala Val Phe Asp Glu Ala Asp Pro Val Gly Gly
 1220 1225 1230
 Pro Val Met Glu Leu Glu Gly Leu Val Phe Gln Ser Leu Gly Ala Ser
 1235 1240 1245

Leu Gly Thr Ser Asp Arg Asp Ser Thr Asp Pro Gly Asn Thr Cys Ser
 1250 1255 1260
 Ser Trp His Trp Ala Pro Asp Ile Ser Leu Val Asn Pro Gly Trp Leu
 1265 1270 1275 1280
 Glu Lys Thr Leu Gly Thr Gly Ile Gln Glu His Glu Ile Ser Leu Ile
 1285 1290 1295
 Leu Glu Leu Arg Arg Cys Ser Val His Phe Ile Gln Glu Ala Met Glu
 1300 1305 1310
 Ser Leu Ser Val Gly Asp Val Glu Arg Leu Ser Gly His Leu Ala Lys
 1315 1320 1325
 Phe Tyr Ala Trp Met Gln Lys Gln Leu Ala Cys Ala Gln Asn Gly Glu
 1330 1335 1340
 Leu Gly Pro Glu Ser Ser Ser Trp Thr Arg Asp Ser Glu Gln Ala Arg
 1345 1350 1355 1360
 Cys Ser Leu Arg Ser Arg Val Val Ala Gly Ser Thr Asn Gly Glu Met
 1365 1370 1375
 Ile Cys Arg Leu Gly Ser Val Leu Pro Ala Ile Leu Arg Arg Glu Val
 1380 1385 1390
 Asp Pro Leu Glu Val Met Met Asp Gly His Leu Leu Ser Arg Tyr Tyr
 1395 1400 1405
 Val Asp Ala Leu Lys Trp Ser Arg Ser Asn Ala Gln Ala Ser Glu Leu
 1410 1415 1420
 Val Arg Leu Cys Cys His Lys Asn Pro Arg Ala Arg Ile Leu Glu Ile
 1425 1430 1435 1440
 Gly Gly Gly Thr Gly Gly Cys Thr Gln Leu Val Val Asp Ser Leu Gly
 1445 1450 1455
 Pro Asn Pro Pro Val Gly Arg Tyr Asp Phe Thr Asp Val Ser Ala Gly
 1460 1465 1470
 Phe Phe Glu Ala Ala Arg Lys Arg Phe Ala Gly Trp Gln Asn Val Met
 1475 1480 1485
 Asp Phe Arg Lys Leu Asp Ile Glu Asp Asp Pro Glu Ala Gln Gly Phe
 1490 1495 1500
 Val Cys Gly Ser Tyr Asp Val Val Leu Ala Cys Gln Val Leu His Ala
 1505 1510 1515 1520
 Thr Ser Asn Met Gln Arg Thr Leu Thr Asn Val Arg Lys Leu Leu Lys
 1525 1530 1535
 Pro Gly Gly Lys Leu Ile Leu Val Glu Thr Thr Arg Asp Glu Leu Asp
 1540 1545 1550
 Leu Phe Phe Thr Phe Gly Leu Leu Pro Gly Trp Trp Leu Ser Glu Glu
 1555 1560 1565
 Pro Glu Arg Gln Ser Thr Pro Ser Leu Ser Pro Thr Met Trp Arg Ser
 1570 1575 1580
 Met Leu His Thr Thr Gly Phe Asn Gly Val Glu Val Glu Ala Arg Asp
 1585 1590 1595 1600

Cys Asp Ser His Glu Phe Tyr Met Ile Ser Thr Met Met Ser Thr Ala
 1605 1610 1615
 Val Gln Ala Thr Pro Met Ser Cys Ser Val Lys Leu Pro Glu Val Leu
 1620 1625 1630
 Leu Val Tyr Val Asp Ser Ser Thr Pro Met Ser Trp Ile Ser Asp Leu
 1635 1640 1645
 Gln Gly Glu Ile Arg Gly Arg Asn Cys Ser Val Thr Ser Leu Gln Ala
 1650 1655 1660
 Leu Arg Gln Val Pro Pro Thr Glu Gly Gln Ile Cys Val Phe Leu Gly
 1665 1670 1675 1680
 Glu Val Glu His Ser Met Leu Gly Ser Val Thr Asn Asp Asp Phe Thr
 1685 1690 1695
 Leu Leu Thr Ser Met Leu Gln Leu Ala Gly Gly Thr Leu Trp Val Thr
 1700 1705 1710
 Gln Gly Ala Thr Met Lys Ser Asp Asp Pro Leu Lys Ala Leu His Leu
 1715 1720 1725
 Gly Leu Leu Arg Thr Met Arg Asn Glu Ser His Gly Lys Arg Phe Val
 1730 1735 1740
 Ser Leu Asp Leu Asp Pro Ser Arg Asn Pro Trp Thr Gly Asp Ser Arg
 1745 1750 1755 1760
 Asp Ala Ile Val Ser Val Leu Asp Leu Ile Ser Met Ser Asp Glu Lys
 1765 1770 1775
 Glu Phe Asp Tyr Ala Glu Arg Asp Gly Val Ile His Val Pro Arg Ala
 1780 1785 1790
 Phe Ser Asp Ser Ile Asn Gly Gly Glu Glu Asp Gly Tyr Ala Leu Glu
 1795 1800 1805
 Pro Phe Gln Asp Ser Gln His Leu Leu Arg Leu Asp Ile Gln Thr Pro
 1810 1815 1820
 Gly Leu Leu Asp Ser Leu His Phe Thr Lys Arg Asn Val Asp Thr Tyr
 1825 1830 1835 1840
 Glu Pro Asp Lys Leu Pro Asp Asp Trp Val Glu Ile Glu Pro Arg Ala
 1845 1850 1855
 Phe Gly Leu Asn Phe Arg Asp Ile Met Val Ala Met Gly Gln Leu Glu
 1860 1865 1870
 Ser Asn Val Met Gly Phe Glu Cys Ala Gly Val Val Thr Ser Leu Ser
 1875 1880 1885
 Glu Thr Ala Arg Thr Ile Ala Pro Gly Leu Ala Val Gly Asp Arg Val
 1890 1895 1900
 Cys Ala Leu Met Asn Gly His Trp Ala Ser Arg Val Thr Thr Ser Arg
 1905 1910 1915 1920
 Thr Asn Val Val Arg Ile Pro Glu Thr Leu Ser Phe Pro His Ala Ala
 1925 1930 1935
 Ser Ile Pro Leu Ala Phe Thr Thr Ala Tyr Ile Ser Leu Tyr Thr Val
 1940 1945 1950

Ala Arg Ile Leu Pro Gly Glu Thr Val Leu Ile His Ala Gly Ala Gly
 1955 1960 1965
 Gly Val Gly Gln Ala Ala Ile Ile Leu Ala Gln Leu Thr Gly Ala Glu
 1970 1975 1980
 Val Phe Thr Thr Ala Gly Ser Glu Thr Lys Arg Asn Leu Leu Ile Asp
 1985 1990 1995 2000
 Lys Phe His Leu Asp Pro Asp His Val Phe Ser Ser Arg Asp Ser Ser
 2005 2010 2015
 Phe Val Asp Gly Ile Lys Thr Arg Thr Arg Gly Lys Gly Val Asp Val
 2020 2025 2030
 Val Leu Asn Ser Leu Ala Gly Pro Leu Leu Gln Lys Ser Phe Asp Cys
 2035 2040 2045
 Leu Ala Arg Phe Gly Arg Phe Val Glu Ile Gly Lys Lys Asp Leu Glu
 2050 2055 2060
 Gln Asn Ser Arg Leu Asp Met Ser Thr Phe Val Arg Asn Val Ser Phe
 2065 2070 2075 2080
 Ser Ser Val Asp Ile Leu Tyr Trp Gln Gln Ala Lys Pro Ala Glu Ile
 2085 2090 2095
 Phe Gln Ala Met Ser Glu Val Ile Leu Leu Trp Glu Arg Thr Ala Ile
 2100 2105 2110
 Gly Leu Ile His Pro Ile Ser Glu Tyr Pro Met Ser Ala Leu Glu Lys
 2115 2120 2125
 Ala Phe Arg Thr Met Gln Ser Gly Gln His Val Gly Lys Ile Val Val
 2130 2135 2140
 Thr Val Ala Pro Asp Asp Ala Val Leu Val Arg Gln Glu Arg Met Pro
 2145 2150 2155 2160
 Leu Phe Leu Lys Pro Asn Val Ser Tyr Leu Val Ala Gly Gly Leu Gly
 2165 2170 2175
 Gly Ile Gly Arg Arg Ile Cys Glu Trp Leu Val Asp Arg Gly Ala Arg
 2180 2185 2190
 Tyr Leu Ile Ile Leu Ser Arg Thr Ala Arg Val Asp Pro Val Val Thr
 2195 2200 2205
 Ser Leu Gln Glu Arg Gly Cys Thr Val Ser Val Gln Ala Cys Asp Val
 2210 2215 2220
 Ala Asp Glu Ser Gln Leu Glu Ala Ala Leu Gln Gln Cys Arg Ala Glu
 2225 2230 2235 2240
 Glu Met Pro Pro Ile Arg Gly Val Ile Gln Gly Ala Met Val Leu Lys
 2245 2250 2255
 Asp Ala Leu Val Ser Gln Met Thr Ala Asp Gly Phe His Ala Ala Leu
 2260 2265 2270
 Arg Pro Lys Val Gln Gly Ser Trp Asn Leu His Arg Ile Ala Ser Asp
 2275 2280 2285
 Val Asp Phe Phe Val Met Leu Ser Ser Leu Val Gly Val Met Gly Gly
 2290 2295 2300

Ala Gly Gln Ala Asn Tyr Ala Ala Ala Gly Ala Phe Gln Asp Ala Leu
 2305 2310 2315 2320
 Ala Glu His Arg Met Ala His Asn Gln Pro Ala Val Thr Ile Asp Leu
 2325 2330 2335
 Gly Met Val Gln Ser Ile Gly Tyr Val Ala Glu Thr Asp Ser Ala Val
 2340 2345 2350
 Ala Glu Arg Leu Gln Arg Ile Gly Tyr Gln Pro Leu His Glu Glu Glu
 2355 2360 2365
 Val Leu Asp Val Leu Glu Gln Ala Ile Ser Pro Val Cys Ser Pro Ala
 2370 2375 2380
 Ala Pro Thr Arg Pro Ala Val Ile Val Thr Gly Ile Asn Thr Arg Pro
 2385 2390 2395 2400
 Gly Pro His Trp Ala His Ala Asp Trp Met Gln Glu Ala Arg Phe Ala
 2405 2410 2415
 Gly Ile Lys Tyr Arg Asp Pro Leu Arg Asp Asn His Gly Ala Leu Ser
 2420 2425 2430
 Leu Thr Pro Ala Glu Asp Asp Asn Leu His Ala Arg Leu Asn Arg Ala
 2435 2440 2445
 Ile Ser Gln Gln Glu Ser Ile Ala Val Ile Met Glu Ala Met Ser Cys
 2450 2455 2460
 Lys Leu Ile Ser Met Phe Gly Leu Thr Asp Ser Glu Met Ser Ala Thr
 2465 2470 2475 2480
 Gln Thr Leu Ala Gly Ile Gly Val Asp Ser Leu Val Ala Ile Glu Leu
 2485 2490 2495
 Arg Asn Trp Ile Thr Ala Lys Phe Asn Val Asp Ile Ser Val Phe Glu
 2500 2505 2510
 Leu Met Glu Gly Arg Thr Ile Ala Lys Val Ala Glu Val Val Leu Gln
 2515 2520 2525
 Arg Tyr Lys Ala
 2530

<210> 11
 <211> 249
 <212> PRT
 <213> Aspergillus terreus

<400> 11
 Met Ala Thr Gln Glu Phe Leu Ser Asp Val Ser Ser Gly Phe Leu Ser
 1 5 10 15
 Ala Glu Ala Ile Arg Tyr Arg Val Lys Thr Gly Val Ser Met Asp Gly
 20 25 30
 Trp Met Lys Arg Gly Tyr Ser Cys Asn Ser Val Arg Thr Asp Asp Lys
 35 40 45
 His His Leu Arg His Leu Thr Asn Ile Gly Leu Asp Thr Pro Pro Cys
 50 55 60
 Pro Lys Ser Leu Pro Ala Ala His Ser Ala Val Ala Ser Cys Leu Thr
 65 70 75 80

Phe Val Pro Pro Asp Pro Cys Glu Asn Trp Glu Ala Leu Gln Val Ala
 85 90 95
 Trp Asp Lys Ala Cys Cys Arg Asn Pro Thr Pro Leu Phe Phe Ile Cys
 100 105 110
 Val Ser Leu Leu Phe Ser Phe Tyr Ser Leu Trp Leu Gln Arg Gly Gly
 115 120 125
 Cys Gly Arg Tyr Gly Gly Leu His Arg Val Ser Lys Val Phe Pro Lys
 130 135 140
 Val Trp Pro Asp Asp Met Asp Ser Gln Leu Pro Ser Arg Leu Gln Thr
 145 150 155 160
 Leu Val Ser Lys Arg Lys Pro Glu Pro Ala Pro Asn Asn Ser Thr Tyr
 165 170 175
 Ile Ser Lys Gly Tyr Ala Thr Phe Phe Asn Gln Phe Ser Leu Pro Ser
 180 185 190
 Val Asp Val Thr Gln Ile Leu Asn Gln Thr Leu Gln His His Asp Val
 195 200 205
 Glu Thr Ile Asn Leu Asp Cys Gly Ser Gly Leu Leu Thr Leu Arg Thr
 210 215 220
 Gln Leu Arg Ile Leu Leu Ile Gly Lys Pro Lys Ile Ile Lys Pro Phe
 225 230 235 240
 Ser Gly Leu Arg Thr Ser Ile Asn Glu
 245

<210> 12
 <211> 742
 <212> PRT
 <213> *Aspergillus terreus*

<400> 12
 Met Glu Ser Ala Glu Leu Ser Ser Lys Arg Gln Ala Phe Pro Ala Cys
 1 5 10 15
 Asp Glu Cys Arg Ile Arg Lys Val Arg Cys Ser Lys Glu Gly Pro Lys
 20 25 30
 Cys Ser His Cys Leu Arg Tyr Asn Leu Pro Cys Glu Phe Ser Asn Lys
 35 40 45
 Val Ala Arg Asp Val Glu Lys Leu Gly Ser Arg Val Gly Asp Ile Glu
 50 55 60
 His Ala Leu Gln Arg Cys Leu Ser Phe Ile Asp Ala His Gln Gly Phe
 65 70 75 80
 Arg Asp Leu Ser Arg Pro Gln Ser Gln Glu Ser Gly Tyr Thr Ser Ser
 85 90 95
 Thr Ser Ser Glu Glu Cys Glu Val Asn Leu Tyr Ser Gly Lys His Thr
 100 105 110
 Ser Pro Thr Glu Glu Asp Gly Phe Trp Pro Leu His Gly Tyr Gly Ser
 115 120 125
 Phe Val Ser Leu Val Met Glu Ala Gln Ala Ala Asn Ala Asn Leu Thr
 130 135 140

Ser Trp Leu Pro Val Asp Met Thr Ser Gly Gln Val Ala Glu Met Val
 145 150 155 160
 Ala Phe Asp Arg Gln Ala Val Ser Ala Val Arg Ser Lys Val Ala Glu
 165 170 175
 Ala Asn Glu Thr Leu Gln Gln Ile Ile Glu Asp Ile Pro Thr Leu Ser
 180 185 190
 Ala Ser Glu Asn Asp Thr Phe Leu Pro Ser Leu Pro Pro Arg Ala Leu
 195 200 205
 Val Glu Pro Ser Ile Asn Glu Tyr Phe Lys Lys Leu His Pro Arg Leu
 210 215 220
 Pro Ile Phe Ser Arg Gln Thr Ile Met Asp Ala Val Glu Ser Gln Tyr
 225 230 235 240
 Thr Ile Arg Thr Gly Pro Pro Asp Leu Val Trp Ile Thr Ser Phe Asn
 245 250 255
 Cys Ile Val Leu Gln Ala Leu Thr Gln Thr Ser Ile Ala Asn Lys Val
 260 265 270
 Val Gly Cys Thr Gly Gln Asp Ile Pro Ile Asp Tyr Met Ile Ile Ser
 275 280 285
 Leu Leu Arg Asn Ile Arg Gln Cys Tyr Asn Arg Leu Glu Thr Leu Val
 290 295 300
 Lys Pro Arg Leu Ser Asn Ile Arg Ala Leu Phe Cys Leu Ala Leu Val
 305 310 315 320
 Ala Met Glu Tyr Phe Asp Phe Ala Ile Phe Leu Thr Ile Phe Ala Gln
 325 330 335
 Val Cys Glu Leu Ser Arg Leu Ile Gly Leu His Leu Thr Thr Thr Thr
 340 345 350
 Pro Pro Thr Glu Asp Gly Ala Val Gly Asp Gln Pro Lys Asp Leu Phe
 355 360 365
 Trp Ser Ile Phe Leu Val Asp Lys His Val Ser Ile Ile Gly Gly Lys
 370 375 380
 Ala Cys Leu Leu Pro Ser Tyr Asp Cys Ser Val Pro Leu Pro Pro Tyr
 385 390 395 400
 Asp Ser Ala Ala Pro Leu Pro Asn Ala Phe Ala Ala Arg Ile Arg Leu
 405 410 415
 Ala Phe Ile Leu Glu Glu Ile Tyr Leu Gly Leu Tyr Ser Ala Lys Ser
 420 425 430
 Ser Lys Met Glu Gln Ser Arg Val Arg Arg Arg Ile Arg Arg Ile Ala
 435 440 445
 Arg Lys Leu Ser Gln Trp His Val Gln His Glu His Val Leu Arg Thr
 450 455 460
 Gly Asp Pro Asn Arg Pro Leu Glu Glu Tyr Ile Cys Ala Thr Gln Leu
 465 470 475 480
 Arg Phe Ala Leu Ser Ser Cys Trp Val Leu Leu His Lys Arg Ile Trp
 485 490 495

Ser Gln Glu Arg Gly Ala Val Cys Leu Gln His Ala Arg Asp Cys Leu
 500 505 510
 Met Leu Phe Lys Gln Leu Cys Asp Gly Cys Lys Ser Gly Phe Ser Asn
 515 520 525
 Phe Asp Ser Ile Val Leu Asn Tyr Ser Leu Ile Ser Phe Met Gly Ile
 530 535 540
 Tyr Val His Ile Val Glu Glu Asp Gln Pro Ile His Ser Gln Asp Met
 545 550 555 560
 Glu Ile Leu Thr Phe Phe Ala Ile Tyr Thr Asn Arg Ser Ala Ser Asn
 565 570 575
 Arg Ser Ser Ala Ser Ile Ser Tyr Lys Leu Ser Gln Val Ala Ser Arg
 580 585 590
 Cys Ser Asp Ile Ala Leu Leu Leu Gln Asn Leu Arg Glu Arg Arg Phe
 595 600 605
 Ile Pro Thr Thr Ile Ser Arg Ser Pro Thr Pro Ser Trp Asn Glu Pro
 610 615 620
 Thr Tyr Met Asp Tyr Asp Val Ala Asn Ala Ser Thr Ser Thr Thr Ser
 625 630 635 640
 Thr Gly Ser Ser Tyr Asn Leu Asn Ile Ser Pro Leu Gly Val Pro Gly
 645 650 655
 Asp Gly Gln Val Trp Asp Ile Tyr Phe Asn Pro Arg Glu Ile Pro Met
 660 665 670
 Asp Gly Thr Ile Ala Thr Pro Ser Glu Asp Ala Thr Gln Asp Leu Leu
 675 680 685
 Ser Asn Asp Ala Gly Gln Cys Leu Gly Phe Pro Asp Phe Ser Leu Gly
 690 695 700
 Ile Asp Asn Phe Ser Asp Phe Pro Leu Gly Ile Asp Met Thr Ser Gln
 705 710 715 720
 Ser Glu Phe Gly Leu Ile Met Glu Glu Asp Ile Ile Arg Tyr Glu Arg
 725 730 735
 Leu Leu Asp Arg Pro Val
 740

<210> 13
 <211> 301
 <212> PRT
 <213> Aspergillus terreus

<400> 13
 Met Glu Ser Lys Val Gln Thr Asn Val Pro Leu Pro Lys Ala Pro Leu
 1 5 10 15
 Thr Gln Lys Ala Arg Gly Lys Arg Thr Lys Gly Ile Pro Ala Leu Val
 20 25 30
 Ala Gly Ala Cys Ala Gly Ala Val Glu Ile Ser Ile Thr Tyr Pro Phe
 35 40 45
 Glu Ser Ala Lys Thr Arg Ala Gln Leu Lys Arg Arg Asn His Asp Val
 50 55 60

Ala Ala Ile Lys Pro Gly Ile Arg Gly Trp Tyr Ala Gly Tyr Gly Ala
 65 70 75 80
 Thr Leu Val Gly Thr Thr Leu Lys Ala Ser Val Gln Phe Ala Ser Phe
 85 90 95
 Asn Ile Tyr Arg Ser Ala Leu Ser Gly Pro Asn Gly Glu Leu Ser Thr
 100 105 110
 Gly Ala Ser Val Leu Ala Gly Phe Gly Ala Gly Val Thr Glu Ala Val
 115 120 125
 Leu Ala Val Thr Pro Ala Glu Ala Ile Lys Thr Lys Ile Ile Asp Ala
 130 135 140
 Arg Lys Val Gly Asn Ala Glu Leu Ser Thr Thr Phe Gly Ala Ile Ala
 145 150 155 160
 Gly Ile Leu Arg Asp Arg Gly Pro Leu Gly Phe Phe Ser Ala Val Gly
 165 170 175
 Pro Thr Ile Leu Arg Gln Ser Ser Asn Ala Ala Val Lys Phe Thr Val
 180 185 190
 Tyr Asn Glu Leu Ile Gly Leu Ala Arg Lys Tyr Ser Lys Asn Gly Glu
 195 200 205
 Asp Val His Pro Leu Ala Ser Thr Leu Val Gly Ser Val Thr Gly Val
 210 215 220
 Cys Cys Ala Trp Ser Thr Gln Pro Leu Asp Val Ile Lys Thr Arg Met
 225 230 235 240
 Gln Ser Leu Gln Ala Arg Gln Leu Tyr Gly Asn Thr Phe Asn Cys Val
 245 250 255
 Lys Thr Leu Leu Arg Asn Glu Gly Ile Gly Val Phe Trp Ser Gly Val
 260 265 270
 Trp Phe Arg Thr Gly Arg Leu Ser Leu Thr Ser Ala Ile Met Phe Pro
 275 280 285
 Val Tyr Glu Lys Val Tyr Lys Phe Leu Thr Gln Pro Asn
 290 295 300

<210> 14
 <211> 490
 <212> PRT
 <213> Aspergillus terreus

<400> 14
 Met Thr Lys Gln Ser Ala Asp Ser Asn Ala Lys Ser Gly Val Thr Ala
 1 5 10 15
 Glu Ile Cys His Trp Ala Ser Asn Leu Ala Thr Asp Asp Ile Pro Pro
 20 25 30
 Asp Val Leu Glu Arg Ala Lys Tyr Leu Ile Leu Asp Gly Ile Ala Cys
 35 40 45
 Ala Trp Val Gly Ala Arg Val Pro Trp Ser Glu Lys Tyr Val Gln Ala
 50 55 60
 Thr Met Ser Phe Glu Pro Pro Gly Ala Cys Arg Val Ile Gly Tyr Gly
 65 70 75 80

Gln Lys Leu Gly Pro Val Ala Ala Ala Met Thr Asn Ser Ala Phe Ile
 85 90 95
 Gln Ala Thr Glu Leu Asp Asp Tyr His Ser Glu Ala Pro Leu His Ser
 100 105 110
 Ala Ser Ile Val Leu Pro Ala Val Phe Ala Ala Ser Glu Val Leu Ala
 115 120 125
 Glu Gln Gly Lys Thr Ile Ser Gly Ile Ala Val Ile Leu Ala Ala Ile
 130 135 140
 Val Gly Phe Glu Ser Gly Pro Arg Ile Gly Lys Ala Ile Tyr Gly Ser
 145 150 155 160
 Asp Leu Leu Asn Asn Gly Trp His Cys Gly Ala Val Tyr Gly Ala Pro
 165 170 175
 Ala Gly Ala Leu Ala Thr Gly Lys Leu Leu Gly Leu Thr Pro Asp Ser
 180 185 190
 Met Glu Asp Ala Leu Gly Ile Ala Cys Thr Gln Ala Cys Gly Leu Met
 195 200 205
 Ser Ala Gln Tyr Gly Gly Met Val Lys Arg Val Gln His Gly Phe Ala
 210 215 220
 Ala Arg Asn Gly Leu Leu Gly Gly Leu Leu Ala His Gly Gly Tyr Glu
 225 230 235 240
 Ala Met Lys Gly Val Leu Glu Arg Ser Tyr Gly Gly Phe Leu Lys Met
 245 250 255
 Phe Thr Lys Gly Asn Gly Arg Glu Pro Pro Tyr Lys Glu Glu Glu Val
 260 265 270
 Val Ala Gly Leu Gly Ser Phe Trp His Thr Phe Thr Ile Arg Ile Lys
 275 280 285
 Leu Tyr Ala Cys Cys Gly Leu Val His Gly Pro Val Glu Ala Ile Glu
 290 295 300
 Asn Leu Gln Arg Arg Tyr Pro Glu Leu Leu Asn Arg Ala Asn Leu Ser
 305 310 315 320
 Asn Ile Arg His Val His Val Gln Leu Ser Thr Ala Ser Asn Ser His
 325 330 335
 Cys Gly Trp Ile Pro Glu Glu Arg Pro Ile Ser Ser Ile Ala Gly Gln
 340 345 350
 Met Ser Val Ala Tyr Ile Leu Ala Val Gln Leu Val Asp Gln Gln Cys
 355 360 365
 Leu Leu Ala Gln Phe Ser Glu Phe Asp Asp Asn Leu Glu Arg Pro Glu
 370 375 380
 Val Trp Asp Leu Ala Arg Lys Val Thr Pro Ser His Ser Glu Glu Phe
 385 390 395 400
 Asp Gln Asp Gly Asn Cys Leu Ser Ala Gly Arg Val Arg Ile Glu Phe
 405 410 415
 Asn Asp Gly Ser Ser Val Thr Glu Thr Val Glu Lys Pro Leu Gly Val
 420 425 430

Lys Glu Pro Met Pro Asn Glu Arg Ile Leu His Lys Tyr Arg Thr Leu
 435 440
 Ala Gly Ser Val Thr Asp Glu Thr Arg Val Lys Glu Ile Glu Asp Leu
 450 455 460
 Val Leu Ser Leu Asp Arg Leu Thr Asp Ile Ser Pro Leu Leu Glu Leu
 465 470 475 480
 Leu Asn Cys Pro Val Lys Ser Pro Leu Val
 485 490

<210> 15
 <211> 488
 <212> PRT
 <213> Aspergillus terreus

<400> 15
 Met Gly Arg Gly Asp Thr Glu Ser Pro Asn Pro Ala Thr Thr Ser Glu
 1 5 10 15
 Gly Ser Gly Gln Asn Glu Pro Glu Lys Lys Gly Arg Asp Ile Pro Leu
 20 25 30
 Trp Arg Lys Cys Val Ile Thr Phe Val Val Ser Trp Met Thr Leu Val
 35 40 45
 Val Thr Phe Ser Ser Thr Cys Leu Leu Pro Ala Ala Pro Glu Ile Ala
 50 55 60
 Asn Glu Phe Asp Met Thr Val Glu Thr Ile Asn Ile Ser Asn Ala Gly
 65 70 75 80
 Val Leu Val Ala Met Gly Tyr Ser Ser Leu Ile Trp Gly Pro Met Asn
 85 90 95
 Lys Leu Val Gly Arg Arg Thr Ser Tyr Asn Leu Ala Ile Ser Met Leu
 100 105 110
 Cys Ala Cys Ser Ala Gly Thr Ala Ala Ala Ile Asn Glu Lys Met Phe
 115 120 125
 Ile Ala Phe Arg Val Leu Ser Gly Leu Thr Gly Thr Ser Phe Met Val
 130 135 140
 Ser Gly Gln Thr Val Leu Ala Asp Ile Phe Glu Pro Val Tyr Arg Gly
 145 150 155 160
 Thr Ala Val Gly Phe Phe Met Ala Gly Thr Leu Ser Gly Pro Ala Ile
 165 170 175
 Gly Pro Cys Val Gly Gly Val Ile Val Thr Phe Thr Ser Trp Arg Val
 180 185 190
 Ile Phe Trp Leu Gln Leu Gly Met Ser Gly Leu Gly Leu Val Leu Ser
 195 200 205
 Leu Leu Phe Phe Pro Lys Ile Glu Gly Thr Ser Glu Lys Val Ser Thr
 210 215 220
 Ala Phe Lys Pro Thr Thr Leu Val Ser Ile Ile Ser Lys Phe Ser Pro
 225 230 235 240
 Thr Asp Val Leu Lys Gln Trp Val Tyr Pro Asn Val Phe Leu Ala Val
 245 250 255

Ser Ala Trp Glu Ile Cys Pro Leu His Leu Leu Glu Thr Lys Cys Ser
 260 265 270

Cys Arg Lys Gln Lys Asp Leu Cys Cys Gly Leu Leu Ala Ile Thr Gln
 275 280 285

Tyr Ser Ile Leu Thr Ser Ala Arg Ala Ile Phe Asn Ser Arg Phe His
 290 295 300

Leu Thr Thr Ala Leu Val Ser Gly Leu Phe Tyr Leu Ala Pro Gly Ala
 305 310 315 320

Gly Phe Leu Ile Gly Ser Leu Val Gly Gly Lys Leu Ser Asp Arg Thr
 325 330 335

Val Arg Arg Tyr Ile Val Lys Arg Gly Phe Arg Leu Pro Gln Asp Arg
 340 345 350

Leu His Ser Gly Leu Ile Thr Leu Phe Ala Val Leu Pro Ala Gly Thr
 355 360 365

Leu Ile Tyr Gly Trp Thr Leu Gln Glu Asp Lys Gly Gly Met Val Val
 370 375 380

Pro Ile Ile Ala Ala Phe Phe Ala Gly Trp Gly Leu Met Gly Ser Phe
 385 390 395 400

Asn Cys Leu Asn Thr Tyr Val Ala Val Glu Ala Leu Pro Arg Asn Arg
 405 410 415

Ser Ala Val Ile Ala Gly Lys Tyr Met Ile Gln Tyr Ser Phe Ser Ala
 420 425 430

Gly Ser Ser Ala Leu Val Val Pro Val Ile Asp Ala Leu Gly Val Gly
 435 440 445

Trp Thr Phe Thr Leu Cys Val Val Ala Ser Thr Ile Ala Gly Leu Ile
 450 455 460

Thr Ala Ala Ile Ala Arg Trp Gly Ile Asn Met Gln Arg Trp Ala Glu
 465 470 475 480

Arg Ala Phe Asn Leu Pro Thr Gln
 485

<210> 16
 <211> 516
 <212> PRT
 <213> Aspergillus terreus

<400> 16
 Met Thr Leu Gln Ile Ile Val Ile Ala Ala Thr Ala Val Ile Tyr Phe
 1 5 10 15

Leu Thr Arg Tyr Phe Asn Arg Thr Asp Ile Pro Lys Ile Lys Gly Ile
 20 25 30

Pro Glu Ile Pro Gly Val Pro Ile Phe Gly Asn Leu Ile Gln Leu Gly
 35 40 45

Val Lys His Ala Thr Val Ala Arg Lys Trp Ser Lys Glu Phe Gly Pro
 50 55 60

Val Phe Gln Ala Arg Leu Gly Asn Arg Arg Val Ile Phe Ala Asn Thr
 65 70 75 80

32

Thr Arg Met Cys Ala Ala Ser His Leu Ala Ser Arg Glu Leu Tyr Thr
435 440 445

Val Phe Leu Arg Phe Ile Val Ala Phe Thr Ile Glu Pro Ala Gln Asn
450 455 460

Pro Ala Asp Met Pro Val Leu Asp Ala Ile Glu Cys Asn Ala Thr Pro
465 470 475 480

Thr Ser Met Thr Thr Glu Pro Lys Pro Phe Lys Val Gly Phe Lys Pro
485 490 495

Arg Asp Glu Thr Ser Leu Arg Arg Trp Ile Ala Glu Ser Glu Glu Arg
500 505 510

Thr Lys Glu Leu
515

<210> 17

<211> 481

<212> PRT

<213> Aspergillus terreus

<400> 17

Met Lys Pro Ala Ile Leu Met Lys Tyr Trp Leu Phe Val Ser Ala Val
1 5 10 15

Ser Ala Ser Thr Leu Asn Gly Lys Leu Thr Leu Ser Glu Thr Lys Val
20 25 30

Thr Gly Ala Val Gln Leu Ala Cys Thr Asn Ser Pro Pro Asp Ile Tyr
35 40 45

Ile Asp Pro Asp Asp Ser Val Ser Val Val Arg Ala Ala His Asp Leu
50 55 60

Ala Leu Asp Phe Gly Arg Val Phe Gly Lys Asn Ala Thr Val Arg Phe
65 70 75 80

Thr Asn Glu Thr His Pro Thr Ser Met Ala Ile Ile Ala Gly Thr Ile
85 90 95

Asp Lys Ser Thr Phe Leu Gln Arg Leu Ile Ala Asp His Lys Leu Asp
100 105 110

Val Thr Ser Ile Arg Gly Gln Trp Glu Ser Tyr Ser Ser Ala Leu Val
115 120 125

Leu Gly Pro Ala Lys Gly Ile Gln Asn Ala Leu Val Ile Ala Gly Ser
130 135 140

Asp Arg Arg Gly Ala Ile Tyr Gly Leu Tyr Asp Ile Ser Glu Gln Ile
145 150 155 160

Gly Val Ser Pro Leu Phe Trp Trp Thr Asp Val Thr Pro Thr Lys Leu
165 170 175

Asp Ala Ile Tyr Ala Leu Asp Val Gln Lys Val Gln Gly Pro Pro Ser
180 185 190

Val Lys Tyr Arg Gly Ile Phe Ile Asn Asp Glu Ala Pro Ala Leu His
195 200 205

Asn Trp Ile Leu Ala Asn Tyr Gly Glu Val Glu Asn Gly Asp Pro Ala
210 215 220

Phe Ile Ser Arg Phe Tyr Ala His Val Phe Glu Leu Ile Leu Arg Leu
 225 230 235 240
 Lys Gly Asn Tyr Leu Trp Pro Ala Met Trp Ser Asn Met Phe Tyr Val
 245 250 255
 Asp Asp Thr Asn Asn Gly Pro Leu Ala Asp Tyr Tyr Gly Val Val Met
 260 265 270
 Gly Thr Ser His Thr Gly Met Thr Val Gly Thr Pro Cys Leu Lys Ala
 275 280 285
 His Ala Asp Tyr Glu Lys Glu Pro Met Ala Arg Ala Thr Asn Glu Gln
 290 295 300
 Ser Gln Phe Leu Asn Gly Thr Trp Asp Trp Ile Ser Asn Glu Val Asn
 305 310 315 320
 Val Lys Ala Phe Met Arg Glu Gly Val Ile Arg Ser Gln His Trp Glu
 325 330 335
 Thr Ala Tyr Thr Met Gly Met Arg Gly Leu Gly Asp Ala Ala Ser Pro
 340 345 350
 Thr Leu Asn Ala Thr Val Glu Glu Ser Ile Val Ser Trp Gln Glu Ser
 355 360 365
 Val Leu Ser Asp Ile Leu Asn Lys Thr Asn Leu Ser Asn Val Val Gln
 370 375 380
 Pro Phe Val Leu Phe Asp Glu Leu Gly Thr Tyr Tyr Glu Ser Gly Met
 385 390 395 400
 Thr Val Pro Asp Gln Val Thr Leu Ile Tyr Pro Asp Asp Asn Ala Gly
 405 410 415
 Asn Met Leu Arg Leu Pro Leu Gln Asn Glu Thr Gly Arg Ser Gly Gly
 420 425 430
 Ala Gly Ile Tyr Tyr His Phe Asp Met Asn Ala Pro Pro Arg Cys Tyr
 435 440 445
 Lys Trp Ile Asn Thr Ala Gln Leu Ile Arg Thr Trp Asp Gln Leu Arg
 450 455 460
 Ala Ala Tyr Ser His Gly Ala Gln Thr Val Trp Val Ala Asn Ile Gly
 465 470 475 480

Asp

<210> 18
 <211> 33000
 <212> DNA
 <213> *Aspergillus terreus*

<400> 18
 tggatttttct tcctgtaggc ccgtagctat gtaatctagc taaacagagc gcgtatttta 60
 aatattagaa actgctcgcg tatcttatcc agagcgtagg ctaggtaggt tacctgggtct 120
 gtttttagcaa gctggacggc ctgcagggcg actaatatatt aggctatttt tataagccccg 180
 gaaagatagc ttatatagct ataaggcttt agaaagatct actgcttaat atctattttct 240
 aaaataataa gaaatctaata aagagtactt ttaaagagat ctttcttaag agtatgggtcg 300
 agtaagataa taaaaaatat taaacaggcc taattaagca gttctttagt ttgctgctgc 360
 tgattaacgc gctacaatag tttaagatct tagctttaga ttaggagatt aactagctgc 420
 cggtataaaa tttttatcta attaaagcgc gtaaaactagg cagtatttag ctagtggcgg 480
 agtaaaatta gctgggttagt ccggctacta tggtaggcga agtaaaataag acactgctag 540

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atctagtagt actaacagta cctcgcctagc cctagataga tctagattag ctggttttag 600
atccccggcc ggccggaagaa gatatataat taaatttagt tgaatttatt aatccggccc 660
ttcttaattg cctaatagct ctaatttaatt agttgcctga cctagcagt aaattacttt 720
agtaaaagatt tagctatagg attagaaaag ctgccagta ggccgactgc ggttttaatt 780
taattataaa cgtattccgc ctattaaata agctctagct ataaggaaat tctagttaga 840
tctagattaa taataagagat ctatgcgtta gtccatccgc cgttagccca attttttat 900
aaagcctgct cgacccgagc ctgaataaatt atagctaaag tctttagaag gaccccttcc 960
tctagtcttt aattlagaaag ggcctcgcct tatatctact taaaqaatta acrtatagat 1020
tagctgataa agaagcgctc atagctaata atatactctg ttagtcgggc gcgaagccca 1080
gcttatattt aagtaataagt cttctaactc tatttctctc gtgccctatt ataattagta 1140
tagttttaat ttttaatttt atttattgct tgtcggcact aatagatata ttataatat 1200
aggcagctat aactacggtg gactggaaga cctaaaatca gagagctact tagaggggg 1260
aaataaataa tctcctactt tagattaaat tagagctgct gaagtattac agttaagca 1320
gcttgttaga gggccggaat agtgggtatt gaataaaagc tataacgcgt ttggaggtag 1380
ataataaaag tagtagaaga aactatatac tagtaggaag gtgtagtata gatctagatc 1440
ttataggtta agttatagag aaagagctct attgttaatt ctaggctcta agagaaaagt 1500
acctagagag ttaagaaata aggaaaatag gtttctataa ggagctattt tcttttaatt 1560
atttattata ttttttaaag atatattaat ggcgcgtcgg atacacgtag taaaaagtaa 1620
attcgtgtct gctattgctt attcctgaag ataaaaata gatataatct cgtagggtcc 1680
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<212> DNA

<213> *Aspergillus terreus*

<400> 20

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<212> DNA

<213> *Aspergillus terreus*

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(74) Agent: BAKER, Jean, C.; Quarles & Brady LLP, 411 East
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(71) Applicant: WISCONSIN ALUMNI RESEARCH
FOUNDATION [US/US]; 614 Walnut Street, Madison,
WI 53705 (US).

(72) Inventors: HUTCHINSON, Richard, C.; 4293
South Deer Run Court, Cross Plains, WI 53528 (US).
KENNEDY, Jonathan; Apartment 102, 401 North Eau
Claire Avenue, Madison, WI 53705 (US). PARK, Cheon-
seok; 11-11 Hwayang-Dong, Kwangjin-ku, Seoul (KR).

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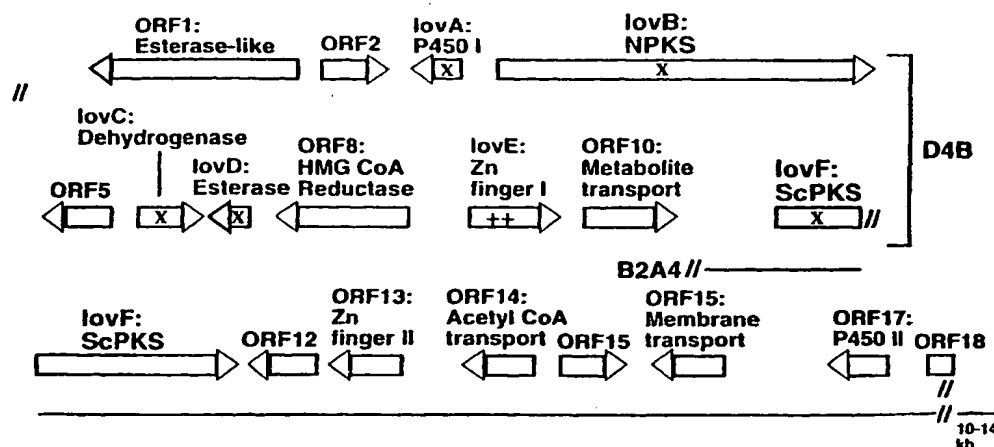
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(54) Title: METHOD OF PRODUCING ANTIHYPERCHOLESTEROLEMIC AGENTS

Lovastatin production genes



(57) Abstract: A method of increasing the production of lovastatin or monacolin J in a lovastatin-producing or non-lovastatin-producing organism is disclosed. In one embodiment, the method comprises the steps of transforming an organism with the *A. terreus* D4B segment, wherein the segment is translated and where an increase in lovastatin production occurs.



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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C12N15/60 C12N1/15 C12N1/19

B. FIELDS SEARCHED

IPC 7 C12N C07K C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, CHEM ABS Data, SCISEARCH, EMBASE, STRAND, GENSEQ, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 744 350 A (DAVIS CHARLES RAY ET AL) 28 April 1998 (1998-04-28) cited in the application	16,17, 24-27
A	claim 6; examples 18,19,27-29	1-11,23, 28,29

X	EP 0 556 699 A (NOVOPHARM LTD) 25 August 1993 (1993-08-25)	28,29
A	claims 1-13; examples 1,2; table 1	1-11, 16-27

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

^o Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
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- *O* document referring to an oral disclosure, use, exhibition or other means
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Date of the actual completion of the international search

4 October 2000

Date of mailing of the international search report

17. 10. 00

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer _____

ALCONADA RODRIG..., A

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/29583

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MANZONI MATILDE ET AL: "Production and purification of statins from <i>Aspergillus terreus</i> strains." BIOTECHNOLOGY TECHNIQUES JULY, 1998, vol. 12, no. 7, July 1998 (1998-07), pages 529-532, XP000921032 ISSN: 0951-208X the whole document	1-11, 16-29
A	WO 97 00962 A (GRAAF LEENDERT H DE ;BROECK H C DEN (NL); PEIJ NOEL N M E (NL); VI) 9 January 1997 (1997-01-09) page 16, line 30 -page 17, line 23 page 18, line 9 -page 24, line 7 SEQ ID NO.9	12-15
A	DATABASE SWISSPROT 'Online! 1 January 1998 (1998-01-01) OLIVER ET AL.: "Putative tricarboxylate transport protein C19G12.05 from fission yeast." XP002149143 Accession 013844	23
A	DATABASE GENEMBL 'Online! 13 May 1997 (1997-05-13) VAN PEIJ ET AL.: "beta-xylosidase, xlnD gene from <i>Aspergillus nidulans</i> " XP002149144 Accession Z84377	23
A	DATABASE SWISSPROT 'Online! 1 October 1996 (1996-10-01) MURPHY ET AL.: "hypothetical 59.3 KDA protein C17C9.16C in chromosome I from <i>Schizosaccharomyces pombe</i> " XP002149145 Accession Q10487	23
P,X	KENNEDY JONATHAN ET AL: "Modulation of polyketide synthase activity by accessory proteins during lovastatin biosynthesis." SCIENCE (WASHINGTON D C) MAY 21, 1999, vol. 284, no. 5418, 21 May 1999 (1999-05-21), pages 1368-1372, XP000914559 ISSN: 0036-8075 the whole document	1-11, 16-29

INTERNATIONAL SEARCH REPORT

In. .ational application No.
PCT/US 99/29583

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-11, 16-22, 24-29 (complete) and 23 (partially)

The D4B gene cluster from *Aspergillus terreus* comprising the ORF1, ORF2, lovA, lovB, ORF5, LovC, lovD, HMG CoA reductase, LovE, ORF10 and part of the lovFA genes involved in the biosynthesis of lovastatin. Uses thereof in a method for increasing the production of lovastatin in a lovastatin-producing organism, for increasing the production of monacolin J in a lovastatin producing organism, and for increasing the production of monacolin J in a non-lovastatin-producing organism; fragments of the D4B gene cluster comprising the gene encoding for the esterase-like gene (ORF1, SEQ ID NO:20), the gene encoding ORF2 (SEQ ID NO:21), the lovA gene (SEQ ID NO:22), the gene encoding ORF5 (SEQ ID NO:23), the lovC gene (SEQ ID NO:24), the lovD gene (SEQ ID NO:25), the gene coding for the HMG CoA reductase (SEQ ID NO:26), the lovE gene (SEQ ID NO:27), the gene encoding ORF10 (SEQ ID NO:28) and the lovB gene (SEQ ID NO:29); a lovastatin-producing organism genetically modified to increase lovastatin production and a non-lovastatin-producing organism genetically modified to produce monacolin J or to produce lovastatin.

2. Claims: 12-15 (complete)

A method of increasing the production of lovastatin in a lovastatin producing organism comprising the step of transforming an organism with the LovE gene from *A.terreus*.

3. Claim : 23 (partially)

An isolated nucleic acid from *Aspergillus terreus* (SEQ ID NO:30) encoding the ORF12 polypeptide (SEQ ID NO:11).

4. Claim : 23 (partially)

An isolated nucleic acid from *Aspergillus terreus* (SEQ ID NO:31) encoding the zinc finger polypeptide of SEQ ID NO:12.

5. Claim : 23 (partially)

An isolated nucleic acid from *Aspergillus terreus* (SEQ ID NO:32) encoding the acetyl-CoA transport polypeptide of SEQ ID NO:13.

6. Claim : 23 (partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

An isolated nucleic acid from *Aspergillus terreus* (SEQ ID NO:33) encoding the ORF15 polypeptide (SEQ ID NO:14).

7. Claim : 23 (partially)

An isolated nucleic acid from *Aspergillus terreus* (SEQ ID NO:34) encoding the membrane transport polypeptide of SEQ ID NO:15.

8. Claim : 23 (partially)

An isolated nucleic acid from *Aspergillus terreus* (SEQ ID NO:35) encoding the P450 polypeptide of SEQ ID NO:16.

9. Claim : 23 (partially)

An isolated nucleic acid from *Aspergillus terreus* (SEQ ID NO:36) encoding the ORF18 polypeptide (SEQ ID NO:17).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/29583

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